Attorney Docket No. 04995.0005-05000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: U.S. Patent No. 5,693,326

Inventors: Andrew Lees, James J. Mond, and Clifford M. Snapper

Assignee: The Henry M. Jackson Foundation for the Advancement of Military Medicine

Title: PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS

Issue Date: December 2, 1997

RATIFICATION OF REQUEST FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Mail Stop: **Hatch-Waxman PTE**
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Sir:

This paper is submitted pursuant to 37 C.F.R. § 1.4(h) and serves to ratify the submission on August 9, 2012, of a Request for Extension of Patent Term for U.S. Patent No. 5,693,326 under 35 U.S.C. § 156. The submission was filed on behalf of The Henry M. Jackson Foundation for the Advancement of Military Medicine but was unsigned. This ratification is submitted by Anthony C. Tridico, an attorney for the law firm Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, which has been appointed under a power of attorney to act for the patent owner for the purpose of filing this Request.

Please charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

Dated: August 27, 2012

Anthony C. Tridico
Reg. No. 45,958
(202) 408-4000

Attorney Docket No. 04995.0005-05000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: U.S. Patent No. 5,693,326

Inventors: Andrew Lees, James J. Mond, and Clifford M. Snapper

Assignee: The Henry M. Jackson Foundation for the Advancement of Military Medicine

Title: PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS

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REQUEST FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Mail Stop: **Hatch-Waxman PTE**
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Sir:

The Henry M. Jackson Foundation for the Advancement of Military Medicine ("Foundation") hereby requests an extension of the term of U.S. Patent No. 5,693,326 ("the '326 patent") pursuant to 35 U.S.C. § 156. A copy of the '326 patent is attached as Exhibit A. An assignment of the '326 patent from the inventors to the Uniformed Services University of the Health Sciences was recorded at reel 010822, frame 0353, on May 19, 2000 and an assignment from the Uniformed Services University of the Health Sciences to the Foundation was recorded at reel 010822, frame 0375, on May 19, 2000. Copies of the recorded assignments are attached as Exhibit B.

The marketing applicant for the approved product underlying this application was GlaxoSmithKline Biologicals SA (GSK). GSK has authorized the Foundation to act as their agent for the purpose of seeking this patent term extension. GSK has also licensed the '326 patent from the Foundation.

A total of five copies of this Request are submitted in compliance with 37 C.F.R. § 1.740(b) and as suggested by MPEP § 2753.

As permitted by 37 C.F.R. § 1.785(b) and M.P.E.P. § 2761, the Foundation is concurrently filing a request for patent term extension of U.S. Patent No. 5,955,079 based upon the same regulatory review period.

The following information is submitted in accordance with 35 U.S.C. § 156(d) and 37 C.F.R. § 1.740, and follows the numerical format set forth in 37 C.F.R. § 1.740(a):

(1) A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics.

The approved vaccine will be marketed under the trademark MENHIBRIX™ and contains three polysaccharide-protein conjugate components — *Neisseria meningitidis* serogroup C capsular polysaccharide (PSC), *Neisseria meningitidis* serogroup Y capsular polysaccharide (PSY), and *Haemophilus influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate, PRP), each covalently bound to tetanus toxoid (TT) using 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP). The vaccine is formulated as a lyophilized product that is reconstituted prior to intramuscular injection using a liquid saline diluent. The reconstituted product contains 2.5 μg of PRP-TT, 5 μg PSC-TT and 5 μg PSY-TT per 0.5 mL dose volume. A copy of the approved package insert for MENHIBRIX ™ is attached as Exhibit D.

(2) A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred.

The regulatory review for MENHIBRIX ™ occurred under Section 351 of the Public Health Service Act (PHSA), which is codified at 42 U.S.C. § 262. Section 351 (42 U.S.C. § 262) provides for the submission and approval of a Biologics License Application (BLA).

(3) An identification of the date when the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred.

MENHIBRIX™ received permission for commercial marketing from the Food and Drug Administration (FDA) pursuant to Section 351 of the PHSA (42 U.S.C. § 262) on June 14, 2012. A copy of the letter from the FDA approving the marketing of MENHIBRIX™ is attached as Exhibit E.

(4) In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.

The approved vaccine contains three active ingredients: PSC-TT, PSY-TT, and PRP-TT. This combination was not previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act prior to the approval of MENHIBRIX™ on June 14, 2012.

(5) A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted.

MENHIBRIX™ was approved for commercial marketing on June 14, 2012. The sixty day period expires on Sunday, August 12, 2012, assuming that June 14, 2012 is the first day of the sixty day period. The present application, therefore, is timely filed within the sixty day period.

(6) A complete identification of the patent for which an extension is being sought by the name of the inventors, the patent number, the date of issue, and the date of expiration.

Inventors: Andrew Lees, James J. Mond, and Clifford M. Snapper

Patent No.: 5,693,326

Issue Date: December 2, 1997

Expiration Date: July 29, 2014

(7) A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings.

A copy of the '326 patent is attached as Exhibit A.

(8) A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent.

A terminal disclaimer has been filed for this patent, disclaiming the portion of the '326 patent that extends beyond the expiration date of U.S. Patent No. 5,651,971. A certificate of correction has also been filed, as well as a certificate for correction of inventorship under 35 U.S.C. § 256 adding James J. Mond and Clifford Snapper as inventors. The 3½, 7½, and 11½ year maintenance fees for the '326 patent have been timely paid.

A copy of the terminal disclaimer, certificate of correction, certificate for correction of inventorship, and receipt showing payment of the maintenance fees are attached as Exhibit F.

(9) A statement that the patent claims the approved product, or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on:

- **(i) The approved product, if the listed claims include any claim to the approved product;**
- **(ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product; and**
- **(iii) The method of manufacturing the approved product, if the listed claims include any claim to the method of manufacturing the approved product.**

The '326 patent claims methods of preparing and using the conjugates in the approved product, MENHIBRIX™. Each applicable patent claim is set forth below together with a showing of the manner in which each applicable patent claim reads on the approved product.

> 1. In a method for preparing a vaccine comprising an immunogenic construct and a pharmaceutically acceptable carrier, the improvement comprising producing the immunogenic construct by a process comprising:
>
> (a) activating a viral, fungal or bacterial polysaccharide with an organic cyanylating reagent selected from the group consisting of 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate, N-cyanotriethyl-ammonium tetrafluoroborate, and p-nitrophenylcyanate, to form an activated carbohydrate; and
>
> (b) coupling said activated carbohydrate directly or indirectly to a protein to form the immunogenic construct capable of stimulating an immune response.

The approved product contains bacterial polysaccharides (PSC, PSY, and PRP), each of which is conjugated to a protein (TT) by activating the bacterial polysaccharides with 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate (CDAP). *See* Exhibit D (Approved Package Insert).

> 2. A method according to claim 1, wherein said organic cyanylating reagent is 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate..

The conjugates in the approved product are prepared using CDAP.

> 3. A method according to claim 2, wherein said polysaccharide and said protein are soluble in water.

The conjugates in the approved product are dissolved and administered in sterile saline solution. *See* Exhibit D (Approved Package Insert).

> 8. A method according to claim 1, wherein the polysaccharide is selected from the group consisting of dextran, Pneumococcal polysaccharide, Haemophilus influenzae polysaccharide, Group A streptococcus polysaccharide, Group B streptococcus polysaccharide, and N. meningitidis polysaccharide.

The conjugates in the approved product are *Neisseria meningitidis* serogroup C capsular polysaccharide, *Neisseria meningitidis* serogroup Y capsular polysaccharide, and *Haemophilus influenzae* type b capsular polysaccharide. *See* Exhibit D (Approved Package Insert).

> 9. A method according to claim 1, wherein the polysaccharide is a water-soluble viral or bacterial polysaccharide.

The conjugates in the approved product are dissolved and administered in sterile saline solution. *See* Exhibit D (Approved Package Insert).

> 10. A method according to claim 1, wherein the protein is a water-soluble protein.

The conjugates in the approved product are dissolved and administered in sterile saline solution. *See* Exhibit D (Approved Package Insert).

> 11. A method according to claim 1, wherein the protein is selected from the group consisting of bovine serum albumin, pertussis toxoid, tetanus toxoid, malaria-derived peptide, an antibody, a toxoid, and a lipoprotein.

The conjugates in the approved product each comprise tetanus toxoid. *See* Exhibit D (Approved Package Insert).

> 13. A method for producing an immune response in a patient comprising:
>
> (a) preparing a vaccine comprising an immunogenic construct capable of stimulating an immune response and a pharmaceutically acceptable carrier, wherein the immunogenic construct is produced by; (i) activating a viral, fungal or bacterial polysaccharide with an organic cyanylating reagent selected from the group consisting of 1-cyano-4-

> (dimethylamino)-pyridinium tetrafluoroborate, N-cyanotriethyl-ammonium tetrafluoroborate, and p-nitrophenylcyanate, to form an activated carbohydrate, and (ii) covalently joining said activated carbohydrate to a protein to form the immunogenic construct; and
>
> (b) administering the vaccine to said patient.

The approved product is administered to a patient to induce an immune response, and the conjugates in the approved product comprise bacterial polysaccharides (PSC, PSY, and PRP), each of which is conjugated to a protein (TT) by activating the bacterial polysaccharides with 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate (CDAP). *See* Exhibit D (Approved Package Insert).

> 14. A method according to claim 13, wherein said organic cyanylating reagent is 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate.

The conjugates in the approved product are prepared using CDAP.

> 16. A method according to claim 14, wherein the protein is a water-soluble protein.

The conjugates in the approved product are dissolved and administered in sterile saline solution. *See* Exhibit D (Approved Package Insert).

> 17. A method according to claim 14, wherein the polysaccharide is selected from the group consisting of dextran, Pneumococcal polysaccharide, Haemophilus influenzae polysaccharide, Group A streptococcus polysaccharide, Group B streptococcus polysaccharide, and N. meningitidis polysaccharide.

The conjugates in the approved are *Neisseria meningitidis* serogroup C capsular polysaccharide, *Neisseria meningitidis* serogroup Y capsular polysaccharide, and *Haemophilus influenzae* type b capsular polysaccharide. *See* Exhibit D (Approved Package Insert).

> 18. A method according to claim 14, wherein the protein is selected from the group consisting of bovine serum albumin, pertussis toxoid, tetanus toxoid, malaria-derived peptide, an antibody, a toxoid, and a lipoprotein.

The conjugates in the approved product each comprise tetanus toxoid. *See* Exhibit D (Approved Package Insert).

(10) A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. 156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:

(i) For a patent claiming a human drug, antibiotic, or human biological product:

(A) The effective date of the investigational new drug (IND) application and the IND number;

(B) The date on which a new drug application (NDA) application or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and

(C) The date on which the NDA was approved or the Product License issued;

GlaxoSmithKline Biologicals SA (GSK), the marketing applicant, filed an IND application on May 12, 2004 (Exhibit G). The IND application for MENHIBRIX™ was assigned BB-IND No. 11,706. The IND became effective on June 12, 2004, thirty days after the FDA received the IND request from GSK. *See* 21 U.S.C. § 355(i)(2).

The Biologics License Application (BLA) for MENHIBRIX™, BL 125363, was submitted to the FDA on August 12, 2009 (Exhibit I).

BL 125363 was approved by the FDA on June 14, 2012 (Exhibit E).

(11) A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities.

Discussions between GSK and FDA were ongoing throughout the regulatory period. GSK initiated discussions with FDA during a pre-IND meeting on April 9, 2003, and also conducted a pre-Phase 3 teleconference with FDA on June 8, 2005 and a pre-BLA meeting on June 19, 2009.

On May 12, 2004, GSK submitted an IND application for MENHIBRIX™ (Exhibit G). The IND (No. 11,706) became effective on June 12, 2004 (Exhibit H). MENHIBRIX™ was designated as a Fast Track Development program on January 24, 2005.

The first Phase II trial in the U.S. of MENHIBRIX™ was initiated on August 13, 2004 and completed on March 29, 2006. Subsequent Phase II studies were conducted. The results of the phase II studies were supportive of further development, and the first Phase III study was initiated on February 22, 2006.

GSK continued to communicate with FDA during the regulatory period, including providing FDA with notifications of Protocol Amendments and amendments to the Chemistry, Manufacturing and Control, Immunogenicity, and Statistical Analysis Sections of the IND. GSK also consulted with FDA regarding additional studies evaluating the effects of co-administering MENHIBRIX™ with other pediatric vaccines. Further detail regarding important communications and other activities undertaken by GSK during the regulatory period are shown in the chronology of major regulatory review events for MENHIBRIX™ (Exhibit J).

On August 12, 2009, GSK submitted a BLA for MENHIBRIX™, which was assigned BLA number BL 125363 (Exhibit I). On September 23, 2011, GSK received a Complete Response letter from FDA indicating that the agency's review of the MENHIBRIX™ file was complete and that

questions remained to be answered prior to approval. GSK worked with FDA to provide responsive information and the BLA was approved on June 14, 2012 (Exhibit E). Exhibit J provides a chronology of major regulatory review events for MENHIBRIX™.

(12) A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of the extension claimed, including how the length of the extension was determined.

It is the opinion of the Applicant that the '326 patent is eligible for patent term extension under 35 U.S.C. § 156(a). The Applicant claims an extension of 1825 days.

Statement of Eligibility of the Patent for Extension

Under 35 U.S.C. § 156(a)

Section 156(a) provides in relevant part, that the term of a patent which claims a product, a method of using a product, or a method of manufacturing a product shall be extended if (1) the term of the patent has not expired before an application for extension is submitted; (2) the term of the patent has never been extended under 35 U.S.C. § 156(e)(1); (3) the application for extension is submitted by the owner of record of the patent or its agent and in accordance with 35 U.S.C. § 156(d)(1)-(4); (4) the product has been subject to a regulatory review period before its commercial marketing or use; and (5) except for 35 U.S.C. §§ 156(a)(5)(B) and 156(a)(5)(C), the permission for the commercial marketing or use of the product after such regulatory review period is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred.

Each of these elements is satisfied here:

(1) The term of the '326 patent expires on July 29, 2014. This application has, therefore, been submitted before the expiration of the patent term.

(2) The term of the '326 patent has never been extended under 35 U.S.C. § 156(e)(1).

(3) The application is submitted by Anthony C. Tridico, an attorney for the law firm Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, which has been appointed

under a power of attorney to act for the owner of the '326 patent for the purpose of filing this Request (Exhibit C). This application is submitted in accordance with 35 U.S.C. § 156(d) within the sixty-day period beginning June 14, 2012, when the product received permission for marketing under the PHSA and contains the information required under 35 U.S.C. §§ 156(d)(1)(A)-(E).

(4) The product was the subject of BB-IND No. 11,706 (filed on May 12, 2004; effective on June 12, 2004), and BLA 125363 (filed on August 12, 2009 and approved on June 14, 2012). Thus, the product was subject to a regulatory review period under § 351 of the PHSA before its commercial marketing or use.

(5) Finally, the permission for commercial marketing of the approved product after regulatory review under PHSA § 351 is the first permitted commercial marketing of the approved product in the United States. This is confirmed by the absence of any approved BLA under which the approved product could be commercially marketed prior to June 14, 2012.

Statement as to the Length of the Extension Claimed

In Accordance with 37 C.F.R. 1.775

The term of the '326 patent should be extended by 1825 days. The extension was determined according to 37 C.F.R. § 1.775 and the PTO worksheet "Calculation of Length for Patent Term Extension for a Human Drug Product" as follows:

(1)	1887	The number of days in the period beginning on the effective date of the IND (June 12, 2004) and ending on the date the BLA was initially submitted (August 12, 2009). This is the "testing phase" as defined in 37 C.F.R. § 1.775(c)(1).
(2)	1037	The number of days in the period beginning on the date the BLA was initially submitted (August 12, 2009) and ending on the date of BLA approval (June 14, 2012). This is the "approval phase" as defined in 37 C.F.R. § 1.775(c)(2).
(3)	2924	The sum of (1) and (2). This is the regulatory review period as define in 37 C.F.R. § 1.775(c).
(4)	0	The number of days in the approval phase (2) which were on and before issuance of the '326 patent. 37

		C.F.R. § 1.775(d)(1)(i).
(5)	0	The number of days in the approval phase (2) during which the Applicant did not act with due diligence. 37 C.F.R. § 1.775(d)(1)(ii).
(6)	0	The sum of (4) and (5).
(7)	2924	The difference between the regulatory review period (3) and (6). 37 C.F.R. § 1.775(d)(1)(ii).
(8)	0	The number of days of the period of the testing phase (1) which occurred prior to the issuance of the '326 patent. 37 C.F.R. § 1.775(d)(1)(i).
(9)	0	The number of days of the period of the testing phase (1) during which the Applicant failed to act with due diligence 37 C.F.R. § 1.775(d)(1)(ii).
(10)	0	The sum of (8) and (9).
(11)	2924	The difference between the regulatory review period (7) and (10).
(12)	1887	The number of days of the testing phase (1).
(13)	0	The number of days from (10).
(14)	1887	Subtract line (13) from line (12).
(15)	943	One half of (14) 37 C.F.R. § 1.775(d)(1)(iii)[1]
(16)	1981	Subtract line (15) from line (11).
(17)	July 29, 2014	The original expiration date of the '326 patent.
(18)	January 1, 2020	The expiration date of the '326 patent if the original expiration date is extended by the number of days in line (16). 37 C.F.R. § 1.775(d)(2)
(19)	June 14, 2012	The date of approval of the application under § 351 of the PHSA.
(20)	14 years	The limitation of 37 C.F.R. § 1.775(d)(3).
(21)	June 14, 2026	The number of years in (20) plus the date on (19). 37 C.F.R. § 1.775(d)(3).
(22)	January 1, 2020	The earlier of line (18) or line (21)
(23)	July 29, 2014	The original expiration date of the '326 patent.
(24)	5 years	The applicable limitation of 37 C.F.R. § 1.775(d)(5)
(25)	July 29, 2019	The number of years on (24) plus the date on (23).

[1] 37 C.F.R. § 1.775(d)(1) provides that for purposes of subtraction, half days are ignored.

(26)	July 29, 2019	The earlier of line (22) or line (25)
(27)	July 29, 2014	The original expiration date of the '326 patent
(28)	1825	The number of days which is the difference between the date on line (27) and the date on line (26)

(13) A statement that the Applicant acknowledges a duty to disclose to the Commission of Patents and Trademarks and to the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought for the '326 patent by this Request as required by 37 C.F.R. § 1.765.

(14) Prescribed Fee:

Please charge the required fee of $1,120.00 as required under 37 C.F.R. § 1.20(j)(1) to Deposit Account No. 06-0916. The Commissioner is authorized to charge any additional fees to Deposit Account No. 06-0916.

(15) The name, address and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed

Anthony C. Tridico
Finnegan, Henderson, Farabow, Garrett
& Dunner, LLP
901 New York Avenue, NW
Washington, DC 20001-4413
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anthony.tridico@finnegan.com

In view of the foregoing, The Henry M. Jackson Foundation for the Advancement of Military Medicine requests that the Commissioner grant an extension of 1825 days to U.S. Patent No. 5,693,326.

Favorable action is earnestly solicited.

Respectfully submitted,

Dated: August 8, 2012

Anthony C. Tridico /JMJ
Anthony C. Tridico

List of Exhibits

Exhibit A - U.S. Patent No. 5,693,326

Exhibit B - Assignment of the '326 patent from the inventors to the Uniformed Services University of the Health Sciences and from the Uniformed Services University of the Health Sciences to the Foundation

Exhibit C - Power of Attorney Authorizing Anthony C. Tridico to Act on Behalf of the Foundation

Exhibit D - Approved package insert for MENHIBRIX™

Exhibit E - FDA Approval Letter

Exhibit F - Copy of the terminal disclaimer, certificate of correction, certificate for correction of inventorship, and receipt showing payment of the maintenance fees for the '326 patent

Exhibit G - Letter dated May 12, 2004, submitting BB-IND No. 11,706

Exhibit H - Letter from FDA acknowledging May 12, 2004 submission of IND 11,706

Exhibit I - Letter of August 12, 2009, submitting BLA 125363 to FDA

Exhibit J - Chronology of Regulatory Review of MENHIBRIX™

Exhibit A

U.S. Patent No. 5,693,326

US005693326A

United States Patent [19]

Lees

[11] Patent Number: 5,693,326

[45] Date of Patent: *Dec. 2, 1997

[54] **PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS**

[75] Inventor: **Andrew Lees**, Silver Spring, Md.

[73] Assignee: **Henry M. Jackson Foundation for the Advancement of Military Medicine**, Rockville, Md.

[*] Notice: The portion of the term of this patent subsequent to Mar. 22, 2012, has been disclaimed.

[21] Appl. No.: **456,694**

[22] Filed: **Jun. 1, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 408,717, Mar. 22, 1995, which is a continuation-in-part of Ser. No. 124,491, Sep. 22, 1993, abandoned.

[51] **Int. Cl.**[6] **A61K 39/385**; C07K 17/10

[52] **U.S. Cl.** **424/194.1**; 424/178.1; 424/193.1; 424/197.11; 530/403; 530/411

[58] **Field of Search** 424/146.1, 196.11, 424/197.11, 178.1, 193.1; 530/406, 411, 403

[56] **References Cited**

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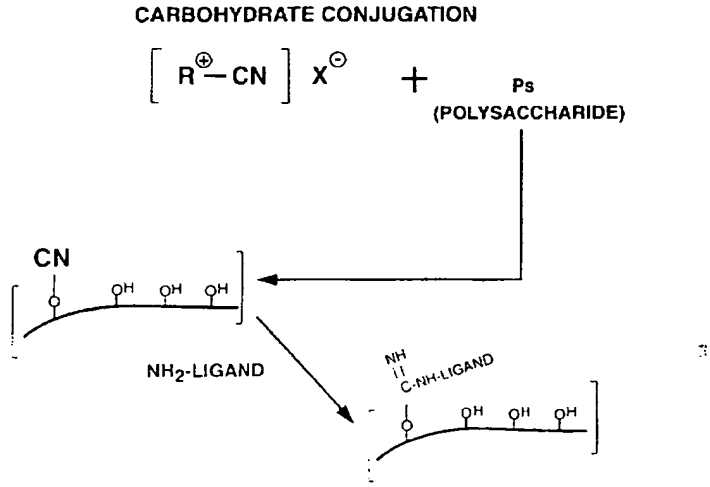
(List continued on next page.)

Primary Examiner—Ponnathapura Achutamurthy
Attorney, Agent, or Firm—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

[57] **ABSTRACT**

The invention relates to a process for producing an immunogenic construct comprising activating at least one first carbohydrate-containing moiety with CDAP, and covalently joining the activated first moiety to a second moiety. Preferably, the first moiety is a polysaccharide and the second moiety is a protein. Immunogenic constructs are prepared by this process using either direct or indirect conjugation of the first and second moieties.

19 Claims, 11 Drawing Sheets



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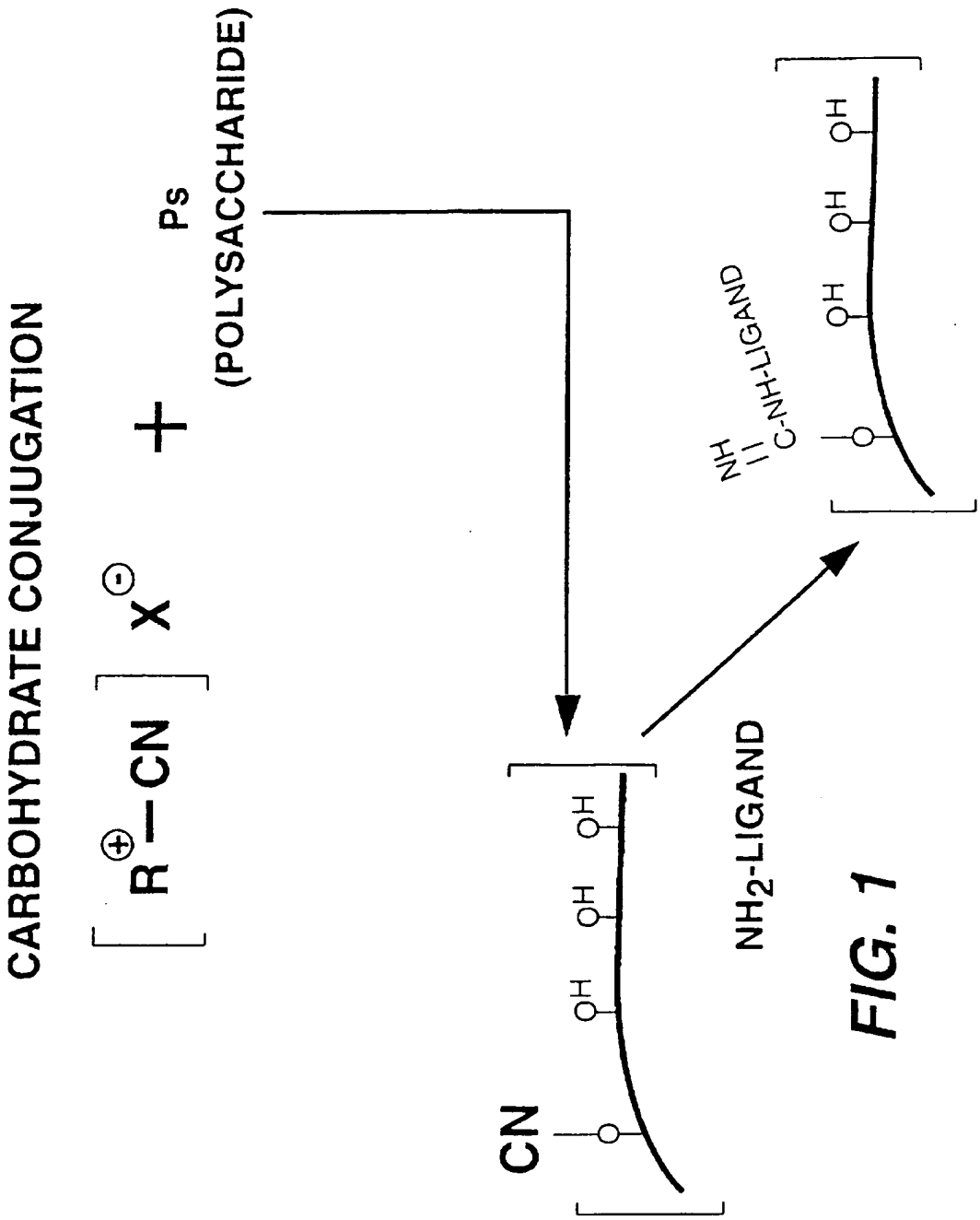
CARBOHYDRATE CONJUGATION
[R⊕—CN] X⊖ + Ps
(POLYSACCHARIDE)
CN
NH2-LIGAND
NH
C-NH-LIGAND
OH

FIG. 1

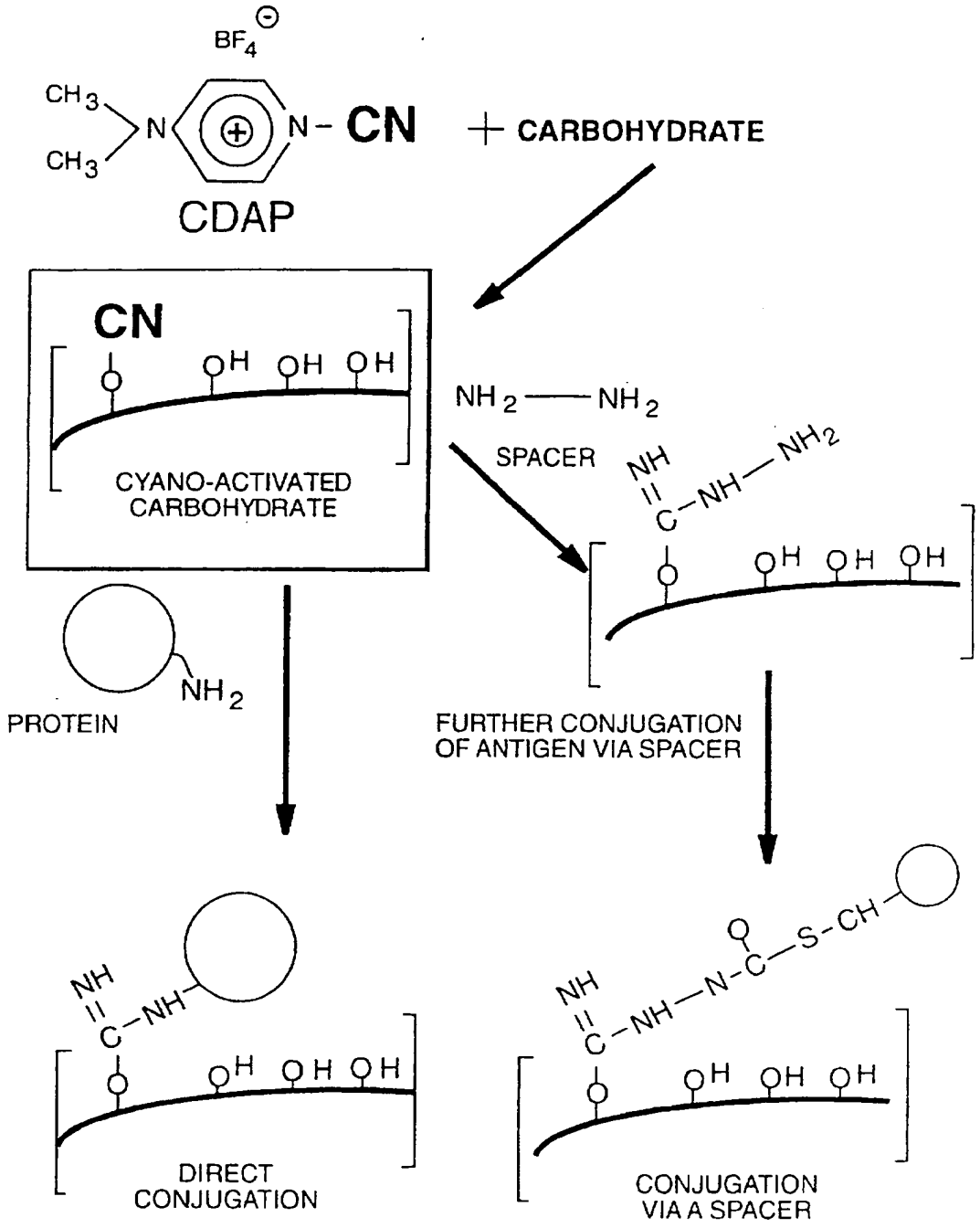
BF4
CH3
CH3
N
N- CN
CDAP
+ CARBOHYDRATE
CN
OH OH OH
CYANO-ACTIVATED CARBOHYDRATE
NH2 — NH2
SPACER
NH
C-NH-NH2
PROTEIN
NH2
FURTHER CONJUGATION OF ANTIGEN VIA SPACER
DIRECT CONJUGATION
CONJUGATION VIA A SPACER

FIG. 2

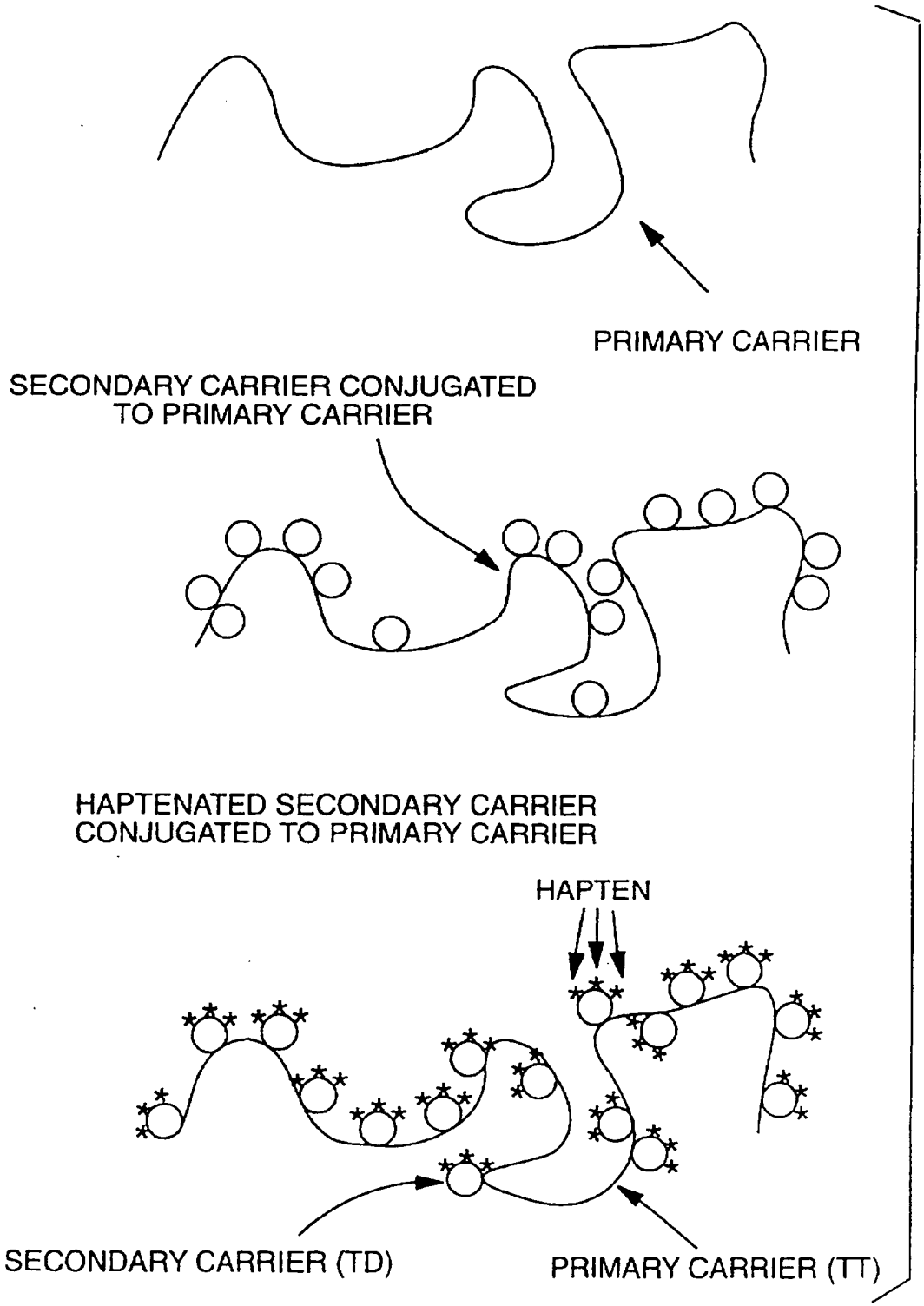
PRIMARY CARRIER
SECONDARY CARRIER CONJUGATED TO PRIMARY CARRIER
HAPTENATED SECONDARY CARRIER CONJUGATED TO PRIMARY CARRIER
HAPTEN
SECONDARY CARRIER (TD)
PRIMARY CARRIER (TT)

FIG. 3

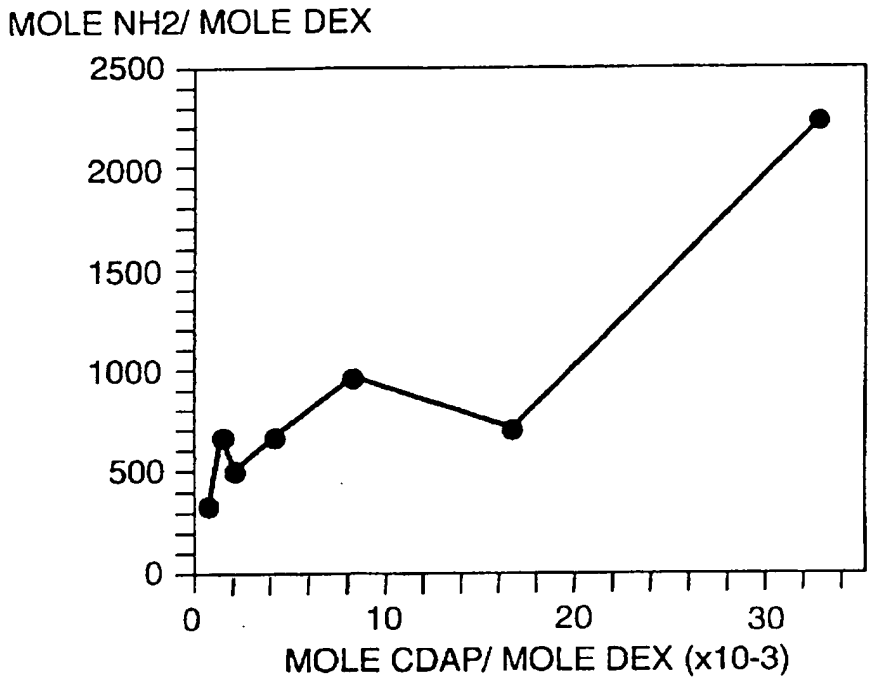
MOLE NH2/ MOLE DEX
2500
2000
1500
1000
500
0
0
10
20
30
MOLE CDAP/ MOLE DEX (x10-3)

FIG. 4

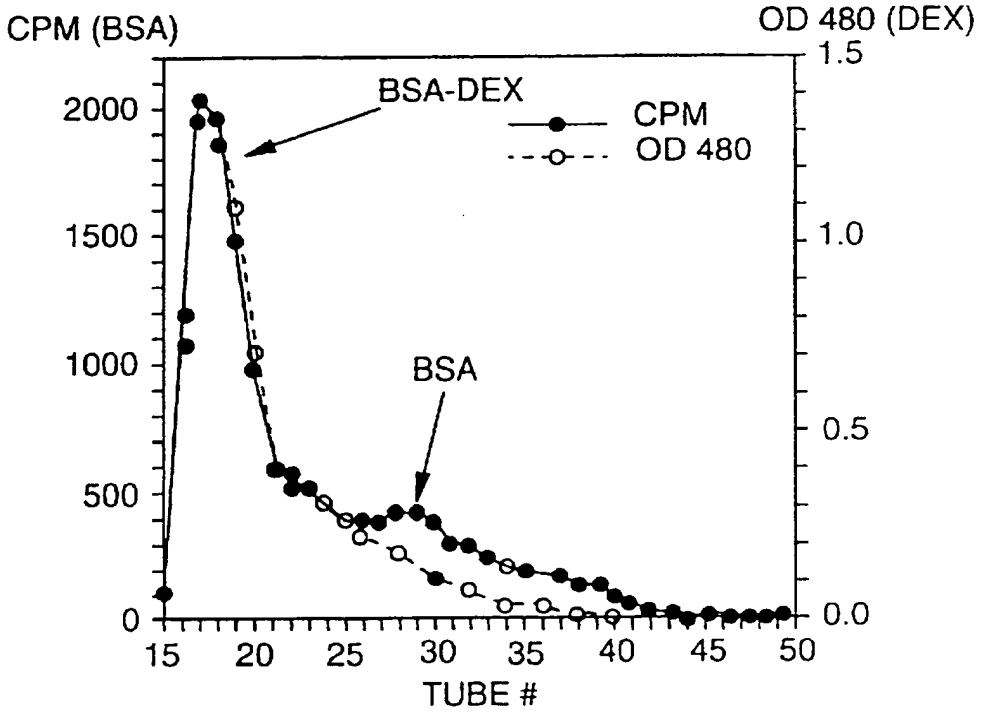
CPM (BSA)
OD 480 (DEX)
BSA-DEX
CPM
OD 480
BSA
2000
1500
1000
500
0
1.5
1.0
0.5
0.0
15
20
25
30
35
40
45
50
TUBE #

FIG. 5

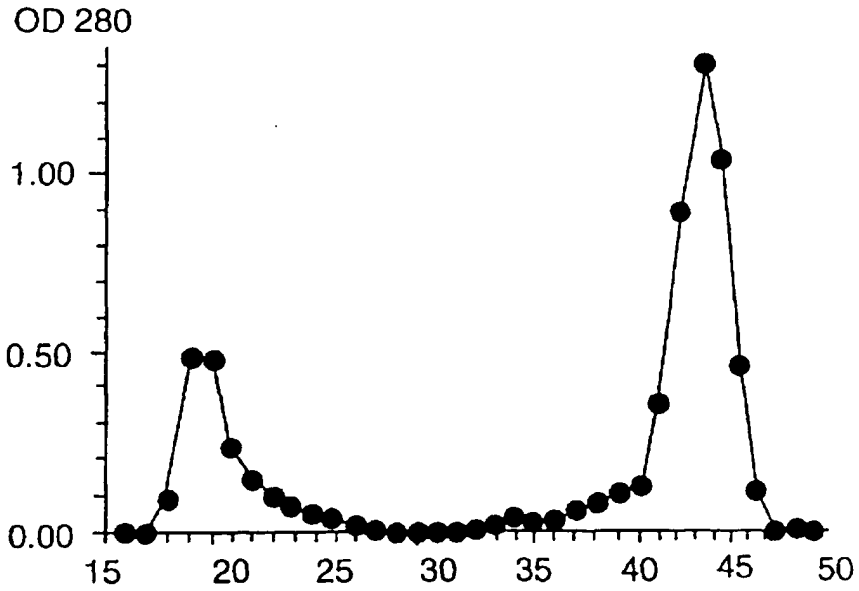
OD 280
1.00
0.50
0.00
15
20
25
30
35
40
45
50
TUBE #

FIG. 6

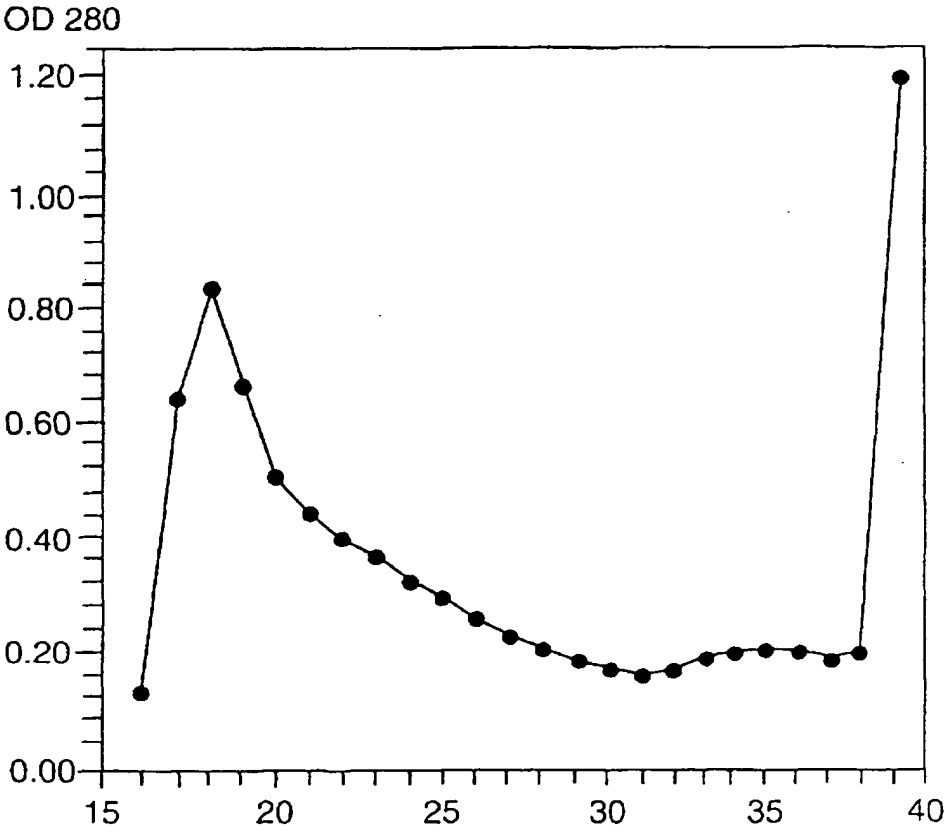
OD 280
1.20
1.00
0.80
0.60
0.40
0.20
0.00
15
20
25
30
35
40
TUBE #

FIG. 7

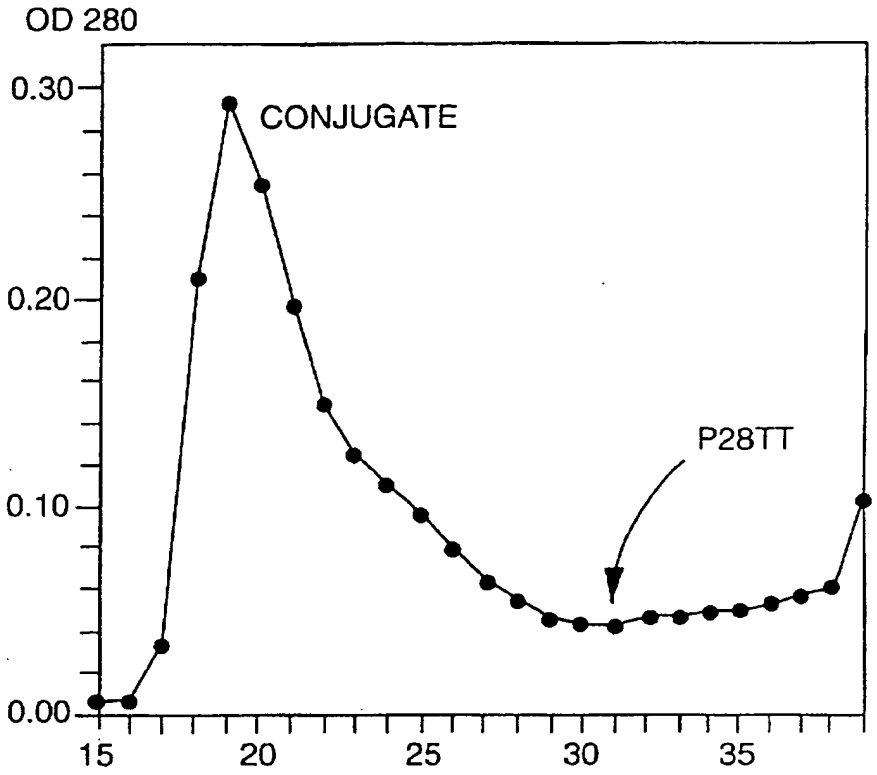
OD 280
0.30
0.20
0.10
0.00
CONJUGATE
P28TT
15
20
25
30
35
TUBE #

FIG. 8

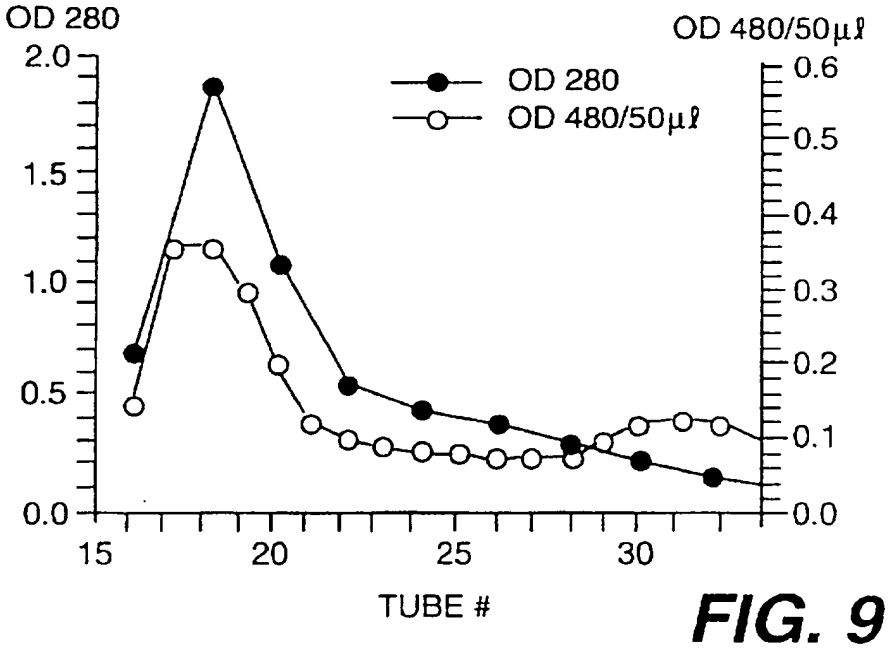
OD 280
OD 480/50µl
2.0
1.5
1.0
0.5
0.0
0.6
0.5
0.4
0.3
0.2
0.1
0.0
OD 280
OD 480/50µl
15
20
25
30
TUBE #

FIG. 9

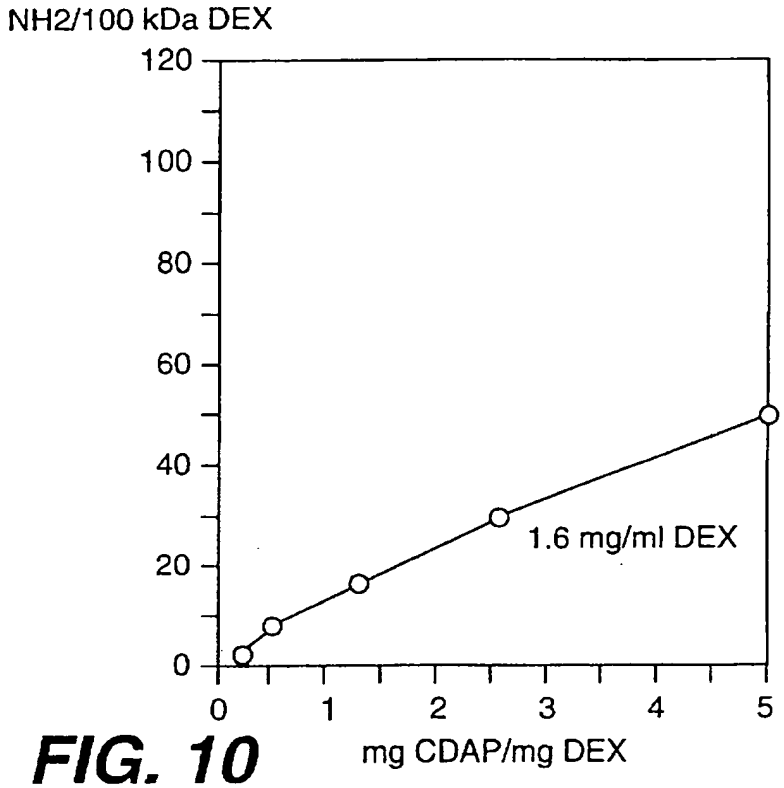
NH2/100 kDa DEX
120
100
80
60
40
20
0
0
1
2
3
4
5
1.6 mg/ml DEX
mg CDAP/mg DEX

FIG. 10

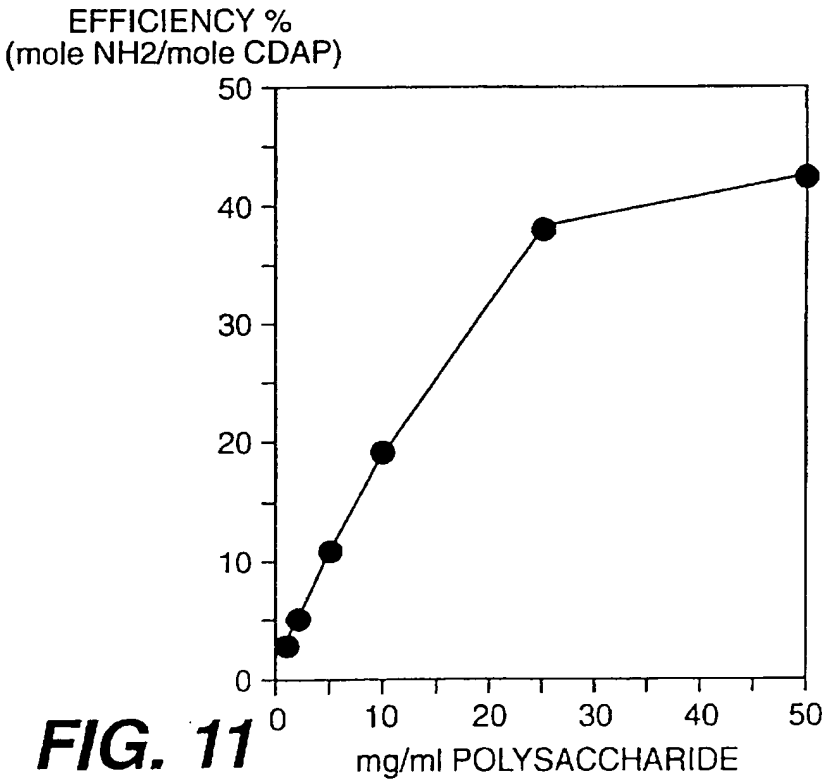
EFFICIENCY %
(mole NH2/mole CDAP)
50
40
30
20
10
0
0
10
20
30
40
50
mg/ml POLYSACCHARIDE

FIG. 11

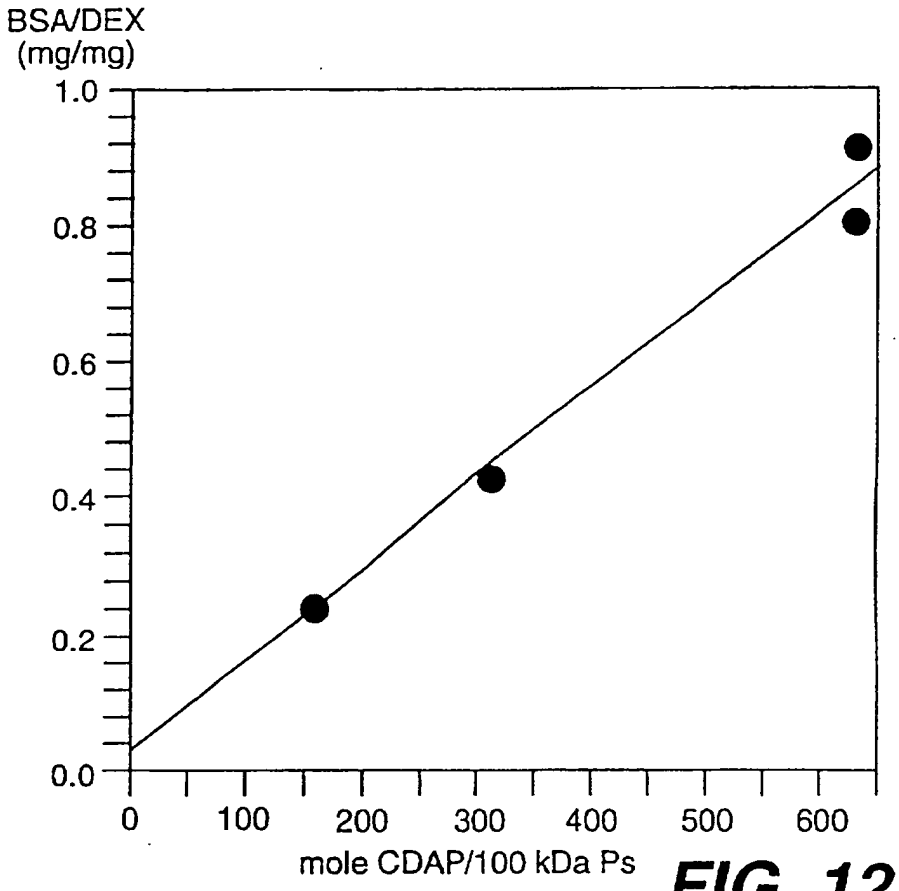
BSA/DEX
(mg/mg)
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
mole CDAP/100 kDa Ps

FIG. 12

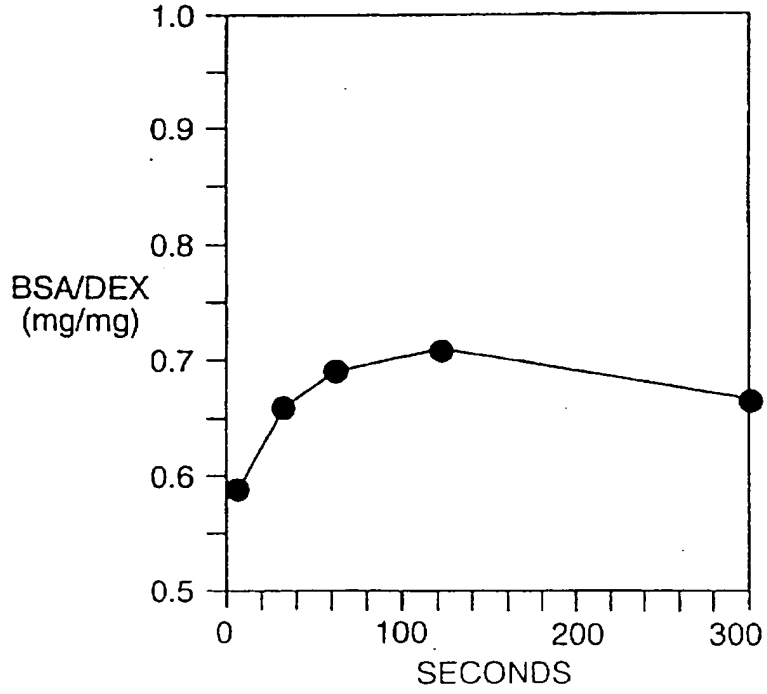
1.0
0.9
0.8
BSA/DEX
(mg/mg)
0.7
0.6
0.5
0
100
200
300
SECONDS

FIG. 13

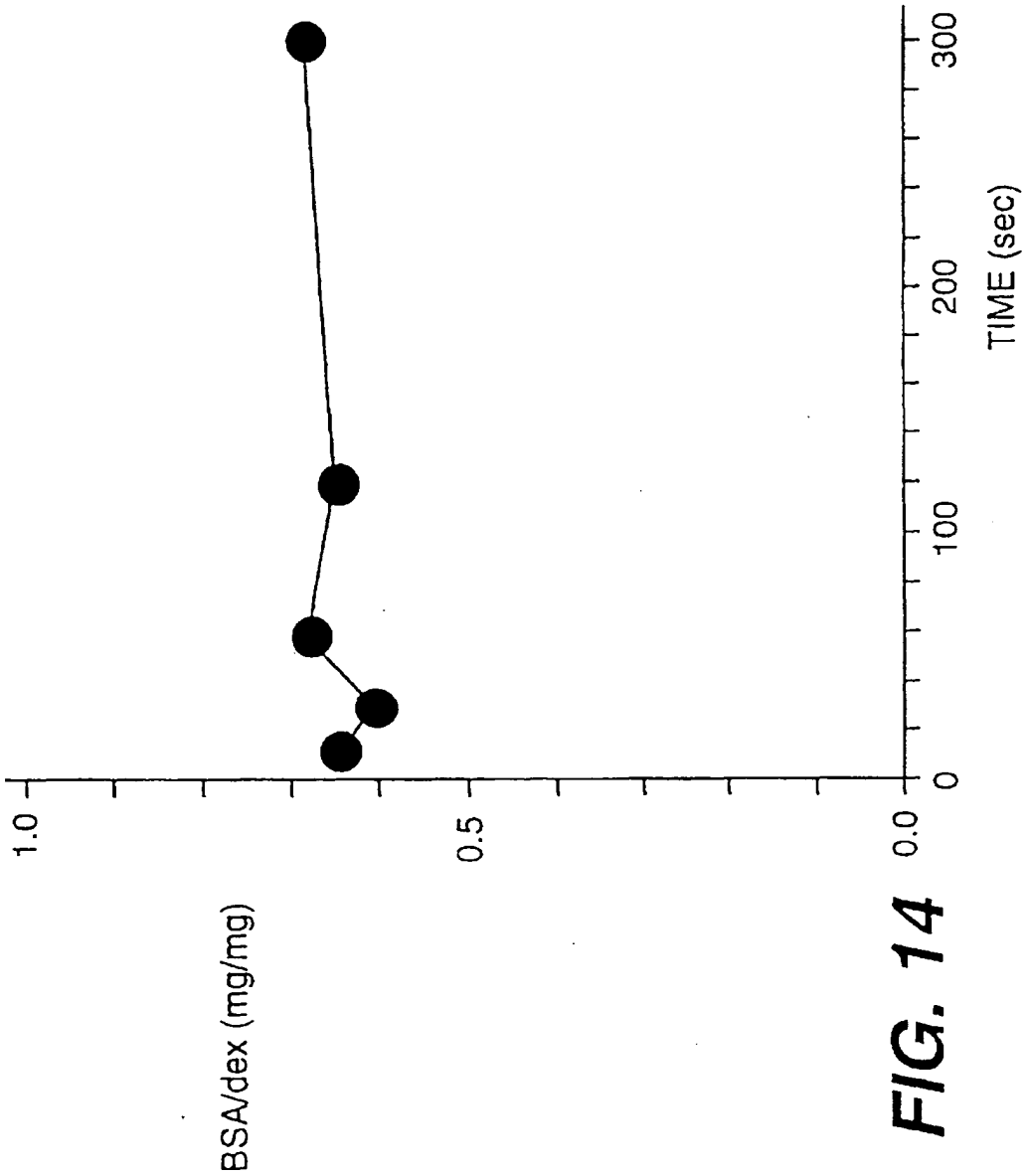

FIG. 14

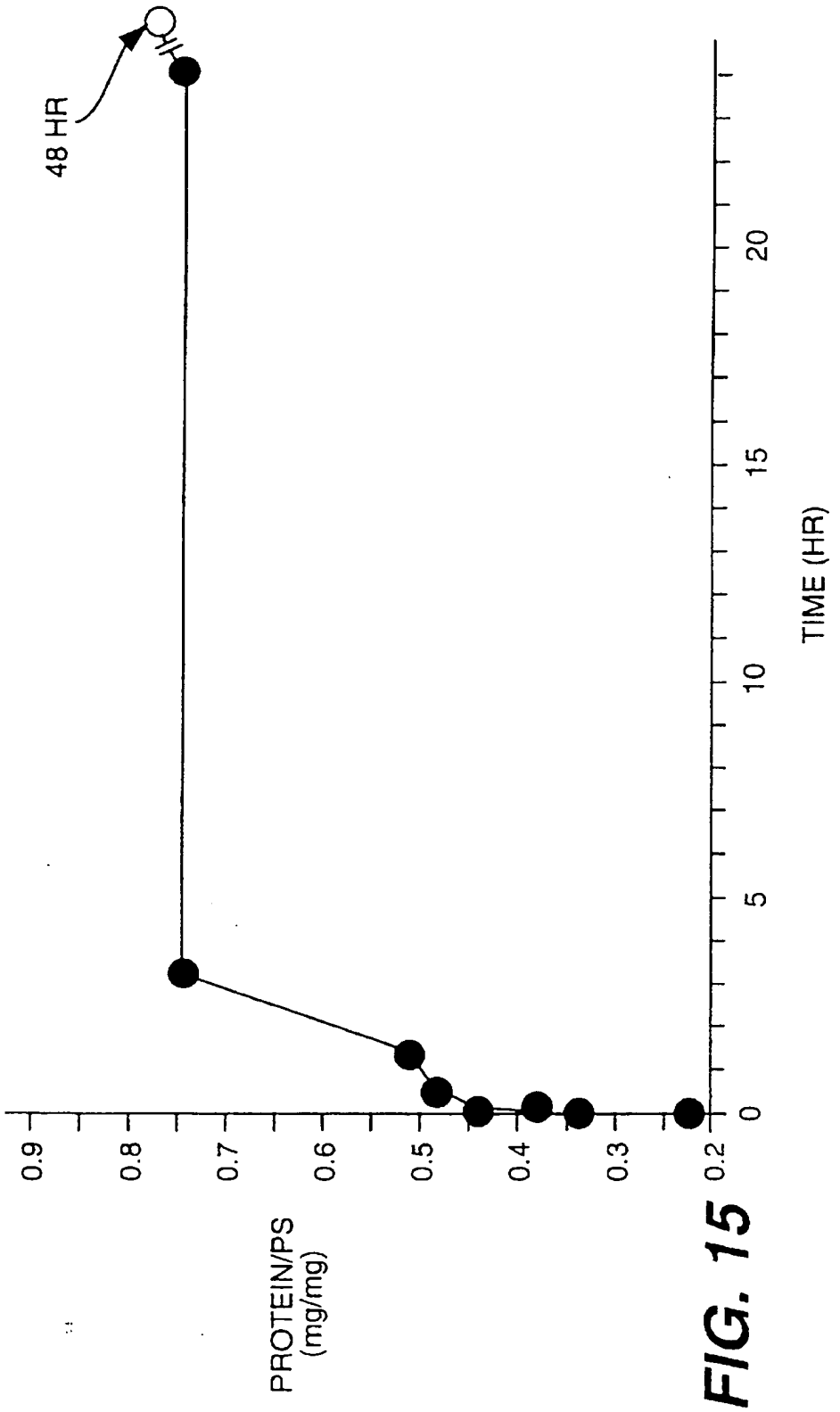

FIG. 15

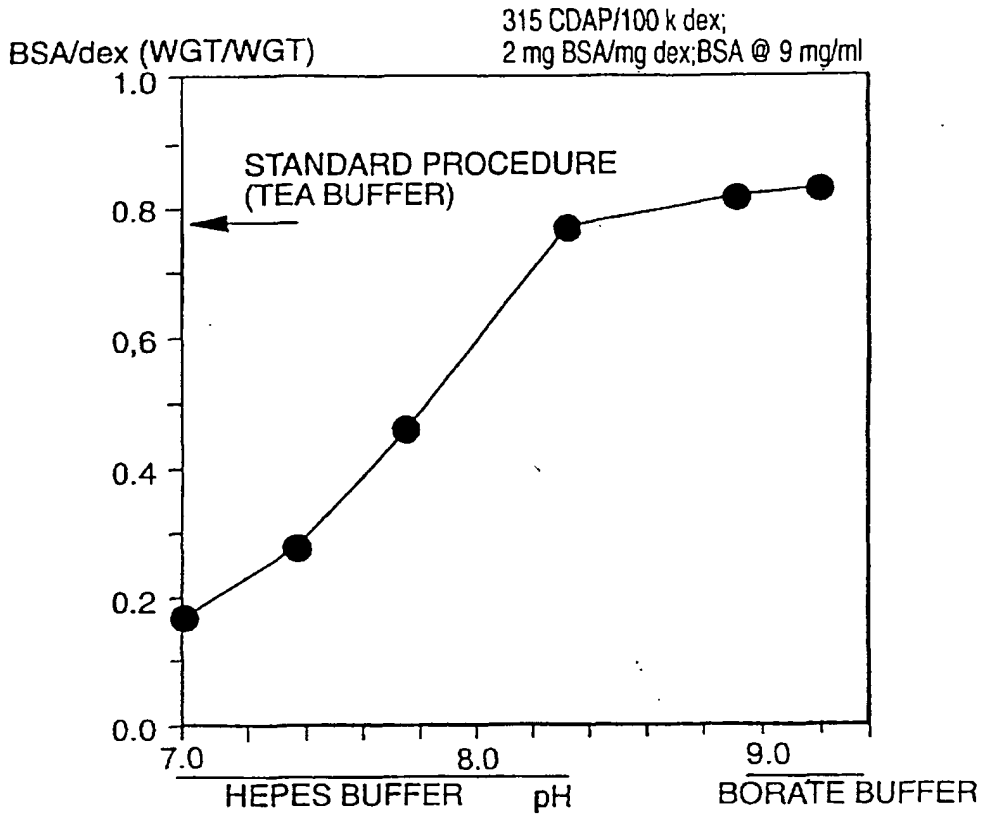
315 CDAP/100 k dex;
2 mg BSA/mg dex;BSA @ 9 mg/ml
BSA/dex (WGT/WGT)
1.0
0.8
0,6
0.4
0.2
0.0
STANDARD PROCEDURE
(TEA BUFFER)
7.0
8.0
9.0
HEPES BUFFER
pH
BORATE BUFFER

FIG. 16

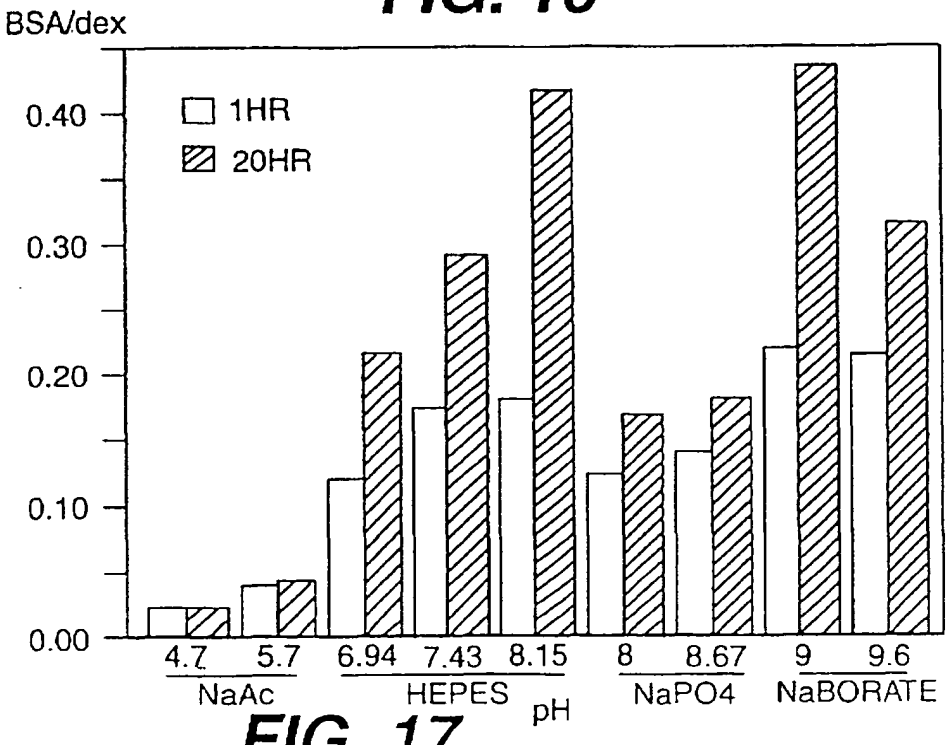
BSA/dex
1HR
20HR
0.40
0.30
0.20
0.10
0.00
4.7
5.7
6.94
7.43
8.15
8
8.67
9
9.6
NaAc
HEPES
pH
NaPO4
NaBORATE

FIG. 17

PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS

This is a continuation of Ser. No. 08/408,717, filed Mar. 22, 1995, which is a continuation-in-part of Ser. No. 08/124,491, filed Sep. 22, 1993, abandoned.

GOVERNMENT INTEREST

The invention may be manufactured, licensed, and used for U.S. governmental purposes without the payment of any royalties to the patent owner thereon.

BACKGROUND OF THE INVENTION

Certain agents such as tetanus toxoid can innately trigger the immune response, and may be administered in vaccines without modification. Other important agents are not immunogenic, however, and must be converted into immunogenic molecules or constructs before they can induce the immune response.

This invention relates generally to advantageous processes for making immunogenic constructs. The invention also relates to the resulting immunogenic constructs and vaccines prepared therefrom, and the use of such immunogenic constructs.

More specifically, the invention relates to methods of activating carbohydrate-containing antigens for use in preparing immunogenic constructs. Immunogenic constructs are very advantageously prepared by activating a carbohydrate-containing moiety with an organic cyanylating agent such as 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate (CDAP).

A variety of cyanylating reagents are known per se, e.g., as reagents for activating insoluble particles to prepare gels for affinity chromatography. See Wilcheck et al., *Affinity Chromatography. Meth. Enzymol.*, 104C:3–55. Wakelsman et al., *J.C.S. Chem. Comm.*, 1976:21 (1976), reported that CDAP is a mild reagent that can be used for modifying protein cysteine groups. Kohn et al., *Anal. Biochem*, 115:375 (1981), compared CDAP, N-cyanotriethyl-ammonium tetrafluoroborate (CTEA), and p-nitrophenylcyanate (pNPC) as activating agents for agarose, an insoluble polysaccharide resin. Other researchers have used CDAP to activate other types of insoluble particles, such as Sepharose and glyceryl-controlled pore glass. See, e.g., Carpenter et al., *Journal of Chromatography*, 573:132–135 (1992).

U.S. Pat. No. 3,788,948 to Kagedal et al. generally describes a method that uses organic cyanate compounds to bind organic compounds containing a primary or secondary amino group to polymers containing one or more hydroxyl and/or primary and/or secondary amino groups, e.g., to bind water-soluble enzymes to water-insoluble polymers. Kagedal et al. describe a method using certain organic cyanate compounds such as pNPC having advantages over cyanogen bromide.

Similarly, Andersson et al., *International Journal of Cancer*, 47:439–444 (1991), report using CDAP to activate a soluble polysaccharide prior to conjugation with protein. They directly conjugated epidermal growth factor (EGF) to low molecular weight 40 kDa dextran activated with cyanate, and used very high dextran to EGF ratios of approximately 50:1 (wt./wt.) to produce dextran-EGF conjugates and studied the binding of this conjugate to cultured cells.

Kagedal et al. and Andersson et al., however, are not concerned with immunogenic constructs. Indeed, conjugates of proteins to low molecular weight dextrans have been reported to be poorly or non-immunogenic. T. E. Wileman, *J. Pharm. Pharmacology*, 38:264 (1985).

The degree of immunogenicity, of course, is an important property of immunogenic constructs for vaccination purposes. The process of vaccination employs the body's innate ability to protect itself against invading agents by immunizing the body with antigens that will not cause the disease but will stimulate the formation of antibodies, cells, and other factors that will protect against the disease. For example, dead organisms are injected to protect against bacterial diseases such as typhoid fever and whooping cough, toxoids are injected to protect against tetanus and diphtheria, and attenuated organisms are injected to protect against viral diseases such as poliomyelitis and measles.

It is not always possible, however, to stimulate antibody formation merely by injecting the foreign agent. The vaccine preparation must be immunogenic, that is, it must be able to induce an immune response. The immune response is a complex series of reactions that can generally be described as follows: (i) the antigen enters the body and encounters antigen-presenting cells that process the antigen and retain fragments of the antigen on their surfaces; (ii) the antigen fragments retained on the antigen-presenting cells are recognized by T cells that provide help to B cells; and (iii) the B cells are stimulated to proliferate and divide into antibody-forming cells that secrete antibodies against the antigen.

Antibodies to most bacterial polysaccharides have been shown to provide protection against infection with encapsulated bacteria. The inability of newborns and infants to mount vigorous responses to T-cell independent (TI) antigens, as exemplified by polysaccharides, has resulted in their extreme susceptibility to life-threatening infections with these organisms. This impaired immune response to TI antigens can be overcome by conjugating T-cell epitopes onto the polysaccharides, thereby converting them into T-cell dependent (TD) antigens.

There are two conjugation methods generally used for producing immunogenic polysaccharide constructs: (1) direct conjugation of carbohydrate and protein; and (2) indirect conjugation of carbohydrates and protein via a bifunctional linker or spacer reagent. Generally, both direct and indirect conjugation require chemical activation of the carbohydrate moiety prior to its derivatization.

Chemical activation refers to the conversion of a functional group to a form that can undergo additional chemical reactions, e.g., the addition of a functional group or of a large moiety such as a protein. Derivatization is the addition of functional chemical group(s) or spacer reagent(s) to a protein.

Unfortunately, artisans have encountered a number of problems in forming immunogenic constructs via conjugation using activation methods. For example, the production of conjugate vaccines has been a formidable challenge, in part, because of the difficulty in activating the polysaccharide and conjugating the protein under conditions that do not lead to their degradation or to the destruction of their immunogenic epitopes. In preparing immunogenic constructs, the method used should be sufficiently gentle to retain important antigenic sites, i.e., epitopes, on the molecules. Thus, it is desirable to maintain the integrity of the structure and to preserve epitopes in these compounds. Unfortunately, the preparation steps currently used in the art are frequently not gentle and can destroy native carbohydrate and/or protein structures.

Moreover, many of the known techniques for carbohydrate modification require anhydrous conditions. Unfortunately, however, carbohydrates are frequently insoluble in organic solvents. Marburg et al., *J. Amer. Chem. Soc.*, 108:5282 (1986).

Thus, although there is a large body of chemical literature describing the modification of carbohydrates, much of it is unsuitable for use with aqueous-based antigens. One approach has been the modification of polysaccharides to enhance their solubility in organic solvents. For example, by replacing the acidic hydrogen on certain acidic polysaccharides with the hydrophobic tetrabutyl ammonium counterion, Marburg et al. were able to solubilize polysaccharides in organic solvents and activate hydroxyls with carbonyl diimidazole, a reagent which must be used in dry solvent. This method is used with polysaccharides, such as *Haemophilus influenzae* PRP and Pneumococcal polysaccharides type 6B and 19F. Coupling of proteins can also be achieved through reductive amination, either using the aldehyde found on the reducing end of the polysaccharide or created by oxidation of the carbohydrate. Both of these approaches have intrinsic limitations and, thus, for high molecular weight polysaccharides, coupling through the reducing end is usually slow and inefficient and oxidation often results in cleavage of the polysaccharide chain or otherwise affects the antigen.

Certain carbohydrates contain groups, such as amino or carboxyl groups, that can be more easily activated or derivatized before conjugation. For instance, the amino groups in Pseudomonas Fisher Type I can be easily derivatized with iodoacetyl groups and bound to a thiolated protein. The carboxyl groups in carbohydrates such as Pneumonococcal type III can be easily activated with water-soluble carbodiimides, such as EDC, and can then be coupled directly to protein. Unfortunately, however, this group of carbohydrates is limited.

Other carbohydrates have aldehyde groups at the terminal reducing end that can be exploited for derivatization and conjugation. It is also possible to create aldehyde groups with oxidizing reagents, e.g., sodium periodate. Aldehyde groups can be condensed with amino groups on protein or with a bifunctional linker reagent. This condensation reaction, especially with the terminal reducing end of a high molecular weight polysaccharide however, often proceeds quite slowly and inefficiently. This is exacerbated when directly conjugating carbohydrate aldehydes to proteins. Thus, yields are often very low using this method. Moreover, sodium periodate may break up carbohydrates into smaller fragments and/or disrupt epitopes, which may be undesirable.

Most carbohydrates must be activated before conjugation, and cyanogen bromide is frequently the activating agent of choice. See, e.g., Chu et al., *Inf. & Imm.*, 40:245 (1983), and Dick & Beurret, "Glycoconjugates of Bacterial Carbohydrate Antigens," *Conjugate Vaccines*, J. M. Cruse & R. E. Lewis (eds.), vol. 10, 48–114 (1989). The first licensed conjugate vaccine was prepared with CNBr to activate HIB PRP, which was then derivatized with adipic dihydrazide and coupled to tetanus toxoid using a water-soluble carbodiimide.

To briefly summarize the CNBr-activation method, cyanogen bromide is reacted with the carbohydrate at a high pH, typically a pH of 10 to 12. At this high pH, cyanate esters are formed with the hydroxyl groups of the carbohydrate. These, in turn, are reacted with a bifunctional reagent, commonly a diamine or a dihydrazide. These derivatized carbohydrates may then be conjugated via the bifunctional group. In certain limited cases, the cyanate esters may also be directly reacted to protein.

The high pH is necessary to ionize the hydroxyl group because the reaction requires the nucleophilic attack of the hydroxyl ion on the cyanate ion (CN^-). As a result, cyanogen bromide produces many side reactions, some of which add neo-antigens to the polysaccharides. M. Wilcheck et al., *Affinity Chromatography. Meth. Enzymol.*, 104C:3–55. More importantly, many carbohydrates or moieties such as HIB PRP and Pn6 can be hydrolyzed or damaged by the high pH necessary to perform the cyanogen bromide activation.

Another problem with the CNBr-activation method is that the cyanate ester formed is unstable at high pH and rapidly hydrolyzes, reducing the yield of derivatized carbohydrate and, hence, the overall yield of carbohydrate conjugated to protein. Many other nonproductive side reactions, such as those producing carbamates and linear imidocarbonates, are promoted by the high pH. Kohn et al., *Anal. Biochem*, 115:375 (1981). Moreover, cyanogen bromide itself is highly unstable and spontaneously hydrolyzes at high pH, further reducing the overall yield.

Furthermore, the cyanogen bromide activation is difficult to perform and unreliable. Cyanogen bromide is highly toxic and potentially explosive. Extreme care must be used when working with large quantities as used in manufacture. All operations must be carried out in a suitable fumehood. It is also known to those in the art that the activation is not easily reproducible because some batches of cyanogen bromide work well and some do not. Cyanogen bromide is also poorly soluble in water, making it difficult to control the amount of soluble cyanogen bromide available to react with the carbohydrate. Even use of the same batch of cyanogen bromide and apparently identical reaction conditions do not always lead to identical results.

In addition to these disadvantages, it is very difficult to control the degree of carbohydrate activation achieved by using cyanogen bromide. It is also very difficult to achieve a high level of carbohydrate activation using this method. Increasing the amount of cyanogen bromide present is ineffective and only leads to increased side reactions without an increase in activation. Kohn et al., *Applied Biochem and Biotech*, 9:285 (1984).

Thus, while cyanogen bromide activation has proven to be a very useful reagent, it has a number of limitations. For example, cyanogen bromide requires a high pH (10–12) in order to make the hydroxyls sufficiently nucleophilic to react with the cyanate ion. However, neither CNBr nor the cyanate ester intermediate is stable at high pH, and consequently most of the reagent either hydrolyzes or undergoes nonproductive or unwanted side reactions. Thus, the efficiency of polysaccharide activation is low. Furthermore, the high pH required for activation can hydrolyze or damage many pH-sensitive polysaccharides. In addition, CNBr is toxic and difficult to work with in small quantities.

Moreover, as noted above, other conjugation methods suffer from various drawbacks. For example, although polysaccharides such as *Cryptococcus neoformans* and Pneumococcal polysaccharide type 3 and VI antigen have carboxyl groups that can be activated with carbodiimides in preparation for coupling to a protein, and polysaccharides such as Pseudomonas Fisher type III have amino groups that can be conveniently used, these antigens form a relatively limited group of all polysaccharides. Other approaches are therefore needed to activate or functionalize the majority of polysaccharides.

Thus, there is a need in the art for a method to produce immunogenic constructs that is gentle, maintains the integrity of the structure of the carbohydrates and proteins, preserves epitopes in the compounds, is easy to perform, is reliable, is readily reproducible, is readily scaled up, and works with a wide variety of polysaccharides.

SUMMARY OF THE INVENTION

An object of the invention is to achieve a gentle method for producing immunogenic constructs. Another object is to arrive at a method for making immunogenic constructs that maintains the integrity of the structure of the carbohydrates

and proteins, and preserves epitopes in the compounds. An additional object is to achieve a method of manufacturing immunogenic constructs that is easy to perform, reliable, and readily reproducible. A further object is to develop a method for making immunogenic constructs that may be used with a variety of polysaccharides. An additional object is to obtain a convenient method for making soluble conjugate vaccines. Another object is to attain a method that is easily scaled up. These and other objects and advantages of the invention will be apparent from the detailed description below.

The present invention attains the above objects, thereby overcoming the problems and disadvantages of known methods for producing immunogenic constructs, by a conjugation process that employs a carbohydrate activation method that is safe, easy, inexpensive, and gentle to carbohydrates. Moreover, the method advantageously employs a homogeneous reaction.

The method of the present invention advantageously uses an organic cyanylating reagent, most preferably 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate (CDAP), to activate carbohydrate-containing moieties. Using the inventive method, a conjugate of a polysaccharide and protein can be prepared where only the polysaccharide is modified, making it possible to recover the protein. Moreover, a conjugate of water-soluble and/or surfactant-soluble moieties may be readily prepared according to the invention.

In one preferred embodiment, the invention comprises directly conjugating the activated carbohydrate-containing moiety to a second moiety, such as a water-soluble protein. In another preferred embodiment, the method of the invention comprises covalently binding a functional (bifunctional or heterofunctional) reagent to the activated carbohydrate-containing moiety, and further reacting the functional reagent with the second moiety, e.g., a T-dependent antigen, to form a conjugate immunogenic construct, wherein the carbohydrate-containing and TD moieties are linked by the spacer or linker formed by the functional reagent.

In another preferred embodiment, the immunogenic construct is a dual-carrier construct of a type described in related U.S. patent application Ser. No. 07/834,067, filed Feb. 11, 1992 (now abandoned), and its continuation-in-part, Ser. No. 08/055,163, filed Feb. 10, 1993 (now abandoned), the specifications of which are incorporated by reference herein. Exemplary primary carriers for such a construct include Pneumococcal type 14 (Pn14) and DNA polymers.

The invention is advantageously applicable to a wide variety of soluble carbohydrate-containing moieties, which after activation with CDAP may be either directly conjugated to protein or indirectly conjugated to protein through a spacer or a linker. The invention enables others to produce more effective immunogenic constructs more efficiently and less expensively than immunogenic constructs prepared using known methods.

Moreover, because CDAP and reaction conditions are so gentle, the risk of destruction of carbohydrate structure and, hence, destruction of naturally-occurring epitopes, is greatly diminished. Furthermore, the method has the advantages summarized in Table 1 below in comparison with the presently used method employing cyanogen bromide.

TABLE 1

Comparison of Carbohydrate Activation in the Synthesis of Conjugates

CNBr	CDAP
High pH (10–12)	Near neutral or mildly basic pH (e.g., 7–9)
Destroys many CHO epitopes	Little of no alteration of CHO epitopes
High toxicity (fume-hood required)	Low toxicity
Dangerous in large quantities	Safe in large quantities
Difficult to work with small quantities	Easy to work with small quantities
Low yields	High yields
Multiple side reactions	Minimal or no side reactions
Does not easily permit direct conjugation to protein	Allows direct conjugation to protein and enables recovery of unconjugated protein
Batch-to-batch variation	Reproducible

Additional advantages to using CDAP are that it can be prepared in advance and stored in a solution for several months, and the concentration of active reagent can be easily determined from its absorbance at 301 nm (Kohn et al., *Anal. Biochem*, 115:375 (1981)). This makes it possible to standardize the reagent concentration and makes the carbohydrate derivatization more reproducible, which is important for its use in vaccine preparation.

The above-mentioned advantages apply both to the direct conjugation of proteins to carbohydrates and to indirect conjugation via a spacer. Additional objects and advantages of the invention will be apparent from the detailed description and the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts an example of a generalized scheme for the activation of carbohydrate using organic cyanylating reagents.

FIG. 2 depicts an exemplary scheme for conjugation of an activated carbohydrate to protein, with direct conjugation shown at the bottom left-hand side and indirect conjugation using a bifunctional reagent shown at the bottom right-hand side.

FIG. 3 shows a model of an immunogenic construct.

FIG. 4 illustrates the incorporation of NH_2 groups into dextran versus the moles of CDAP added per mole of dextran at 10 mg/ml dextran.

FIG. 5 illustrates the elution profile of a ^{3}H-BSA-dextran conjugate from a S400SF gel filtration column.

FIG. 6 illustrates the OD280 absorbance of immunogenic constructs prepared according to the method of the invention, eluted from S400SF gel filtration column.

FIG. 7 illustrates the elution profile of Hδ^a/1-(CDAP)-dextran from S400SF gel filtration column.

FIG. 8 illustrates OD280 and OD430 values of column samples eluted from S400SF gel filtration column loaded with Hδ^a/NH_2-(CDAP)-dextran.

FIG. 9 illustrates the immunoreactivity of immunogenic constructs prepared using the methods of the invention.

FIG. 10 shows the results of derivatization of dextran (dex) with hexane diamine with CDAP (NH_2/100 kDa dex versus mg CDAP/mg dex) at 1.6 mg/ml dextran.

FIG. 11 is a graph of the efficiency of CDAP activation versus the polysaccharide concentration.

FIG. 12 shows the direct conjugation of BSA to dextran for various CDAP:polysaccharide ratios for CDAP activation.

FIG. 13 is a plot of the BSA/dextran ratio versus the time of addition of protein to CDAP-activated dextran.

FIG. 14 shows the stability of CDAP in water.

FIG. 15 illustrates the kinetics of protein coupling to CDAP-activated polysaccharide.

FIG. 16 shows the effect of pH on CDAP activation.

FIG. 17 is a bar graph showing the effect of pH and various buffers on the coupling of BSA to CDAP-activated dextran.

DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

A generalized scheme for the activation of carbohydrates using organic cyanylating reagents (which may be represented generally by the formula R—CN or $\{R^+—CN\}X^-$, where R is an organic moiety and X is a counter-ion) is shown in FIG. 1. FIG. 2 illustrates the conjugation of an activated carbohydrate to protein.

As used herein, "immunogenic construct" refers to an entity that can stimulate the immune response. The immunogenic construct comprises at least one first moiety conjugated to at least one second moiety. As used herein, a "moiety" is any substance that can be used to stimulate the immune system either by itself or upon being coupled.

Exemplary moieties include carbohydrates, synthetic polymers such as polyvinyl alcohol, proteins and glycoproteins, peptides, other antigens, adjuvant molecules, haptens, DNA, and combinations and derivatives thereof. Haptens refer to small molecules, such as chemicals, dust, and allergens, that by themselves are not able to elicit an antibody response, but can once they are coupled to a carrier, e.g., TNP. An antigen is any molecule that, under the right circumstances, can induce the formation of antibodies. These haptens and antigens may derive from but are not limited to bacteria, rickettsiae, fungi, viruses, parasites, drugs, or chemicals. They may include, for example, small molecules such as peptides, oligosaccharides (e.g., the polyribosyl-ribitol-phosphate oligomers of *H. influenzae*), DNA oligomers, lipids, toxoids, endotoxin, etc. Preferred moieties are soluble in water or solubilized in surfactant.

In a preferred embodiment, the first moiety is a carbohydrate-containing moiety. As used herein, "carbohydrate" means any soluble monosaccharide, disaccharide, oligosaccharide, or polysaccharide. Preferably, the first moiety is a polysaccharide, more preferably a water-soluble polysaccharide. Preferred polysaccharides include those listed in the chart below of exemplary vaccines.

The carbohydrate-containing moiety is preferably naturally occurring, a semisynthetic, or a totally synthetic large molecular weight molecule. In a preferred embodiment, at least one carbohydrate-containing moiety is selected from *E. coli* polysaccharides, *S. aureus* polysaccharides, dextran, carboxymethyl cellulose, agarose, Pneumococcal polysaccharides (Pn), Ficoll, *Cryptococcus neoformans, Haemophilus influenzae* PRP, *P. aeroginosa, S. pneumoniae*, lipopolysaccharides, Group A and B streptococcus, *N. meningitidis*, and combinations thereof.

In an especially preferred embodiment, the carbohydrate-containing moiety is a dextran. As used herein, "dextran" (dex) refers to a polysaccharide composed of a single sugar, which may be obtained from any number of sources (e.g., Pharmacia). Another preferred carbohydrate-containing moiety is Ficoll, which is an inert, semisynthetic, non-ionized, high molecular weight polymer.

The carbohydrate-containing moiety is activated using an organic cyanylating reagent. Preferred organic cyanylating reagents are 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate (CDAP), N-cyanotriethylammonium tetrafluoroborate (CTEA), and p-nitrophenylcyanate (pNPC). Of these reagents, CDAP is the most preferred. Other organic complexes with the cyanate group, optionally with a variety of counter-ions, may be used. Particularly preferred organic cyanylating reagents are those with non-nucleophilic counter-ions such as tetrafluoroborate.

After activation via the organic cyanylating reagent, the first moiety is conjugated to the second moiety. Preferably, the second moiety is a protein, which may be selected from viral, bacterial, parasitic, animal, and fungal proteins. Especially preferred second moieties include lipoproteins, bovine serum albumin (BSA), tetanus toxoid (TT), pertussis toxoid (PT), diphtheria toxoid (DT), heat shock protein, T-cell superantigens, and bacterial outer-membrane protein, all of which may be obtained from biochemical or pharmaceutical supply companies or prepared by standard methodologies (see, e.g., J. M. Cruse & R. E. Lewis, (eds.), *Conjugate Vaccines in Contributions to Microbiology and Immunology*, vol. 10 (1989), which is incorporated herein by reference). Other suitable proteins may be selected from those known in the art.

Other preferred embodiments of the second moiety are albumin, a toxoid, a peptide, a T-cell or B-cell adjuvant, or any other compound capable of activating and recruiting T-cell help. The second moiety may be a T-dependent antigen as represented in FIG. 3.

The second moieties of the invention are capable of being conjugated to at least one carbohydrate-containing moiety. The second moieties may either contain functional groups that can react with the carbohydrate-containing moiety or can be chemically manipulated to be capable of reacting with the carbohydrate-containing moiety.

Numerous copies of specific second moieties as well as a variety of second moieties may be conjugated to the carbohydrate-containing moiety. Coupling of multiple copies of the second moiety to the first moiety significantly augments antibody production to the second moiety.

The inventive process allows one to advantageously control the physical and chemical properties of the immunogenic construct. In accordance with the invention, the artisan may advantageously: modify the charge on the first and second moieties (an advantage in light of evidence that cationized proteins may be more immunogenic); control the size of the construct by varying the size of the carbohydrate-containing moiety; select the degree of crosslinking of the inter- and intra-chain construct (to obtain variations of size and of the three-dimensional matrix); control the number of copies of the second moiety conjugated to carbohydrate-containing moieties; and target to selected cell populations (such as to macrophages to enhance antigen presentation). Dick & Beurret, "Glycoconjugates of Bacterial Carbohydrate Antigens," *Conjugate Vaccines*, J. M. Cruse a R. E. Lewis (eds.), vol. 10, 48–114 (1989).

The immune response to the construct of the invention may be further enhanced by the addition of immunomodulators and/or cell-targeting moieties. These entities include, for example, (1) detoxified lipopolysaccharides or derivatives, (2) muramyl dipeptides, (3) carbohydrates, lipids, and peptides that may interact with cell surface determinants to target the construct to immunologically relevant cells, (4) interleukins, (5) antibodies, and (6) DNA oligomers.

Thus, in alternative embodiments, third moieties may be conjugated to one or more of the first and/or second moieties using methods such as CDAP activation as described herein or other known techniques. U.S. patent application Ser. Nos. 07/834,067 and 08/055,163 (both applications now abandoned) describe conjugation that promotes enhanced antibody responses to the third moiety. Certain techniques to

conjugate various moieties to either the first or second moieties are well known to those skilled in the art, e.g., involving coupling through available functional groups (such as amino, carboxyl, thio and aldehyde groups). See S. S. Wong, *Chemistry of Protein Conjugate and Crosslinking CRC Press* (1991), and Brenkeley et al., "Brief Survey of Methods for Preparing Protein Conjugates With Dyes, Haptens and Cross-Linking Agents," *Bioconjugate Chemistry*, 3:1 (January 1992), which are incorporated herein by reference. Thus, monofunctional reagents may be used as third moieties, e.g., to modify the charge, change the hydrophobicity, label the construct, etc.

In the method of the invention, the carbohydrate-containing moiety is activated using an organic cyanylating reagent. The organic cyanylating reagent is preferably CDAP, which increases the electrophilicity of the cyanate and, when reacted with carbohydrate-containing moieties, transfers a cyano group to the hydroxyl groups of the carbohydrate, thus preparing it for further reaction, i.e., direct or indirect conjugation to protein. The activation reaction can be carried out at neutral pH or under mildly basic conditions (e.g., a pH of about 8 to about 10), which improves the stability and integrity of the polysaccharide and the active intermediate.

CDAP is advantageous because it is highly stable and is relatively safe. CDAP is a water-soluble organic cyanylating reagent in which the electrophilicity of the cyano group is increased, advantageously permitting the cyanylation reaction to be performed under mild conditions. Furthermore, CDAP can be used to activate a wide variety of polysaccharides, which can then be functionalized with diamines or dihydrazides. The high levels of activation and mild conditions of the CDAP cyanylation reaction permit proteins to be directly conjugated to polysaccharides in a one-pot reaction, thereby simplifying the preparation of conjugate vaccines that induce antibody responses to both the polysaccharide and the protein components, even in the absence of a spacer molecule. The ease of use of CDAP facilitates the preparation of protein-polysaccharide conjugate vaccines under a variety of conditions, thus making possible the study of the important parameters of the immunogenicity of conjugate vaccines. Moreover, CDAP-activated polysaccharides can be used to prepare a variety of other useful immunological reagents, e.g., biotinylated polysaccharides and antibody-linked dextrans such as $H\delta^a$/1.

The activation is preferably performed at a pH of from about 6 to about 10, more preferably of from about 9 to about 10. The pH may be adjusted by a variety of techniques (e.g., using a buffer, adding NaOH, etc.) to suit the particular construct being prepared. For example, the activation may be carried out in a variety of solvents using one or more of a variety of suitable non-nucleophilic buffers known in the art. Suitable solvents include saline, water, and some organic solvents. Examples of suitable non-nucleophilic buffers include triethyl amine (TEA), 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES), phosphate, carbonate, and borate. Preferably, triethyl amine (TEA) is used as a buffer.

In a preferred embodiment of the invention, CDAP is dissolved in a stock solution at a concentration of 100 mg/ml in dry acetonitrile or up to 75 mg/ml in water. Depending on the nature of the carbohydrate-containing moiety used and the degree of activation desired, various amounts of CDAP may be optimal.

In a preferred embodiment, the concentration of the carbohydrate-containing moiety is from 1 to 20 mg/ml, more preferably from 1 to 15 mg/ml. The activation reaction can be performed successfully with concentrations of carbohydrate-containing moiety up to about 100 mg/ml.

Preferably, the CDAP to carbohydrate-containing moiety ratio for direct conjugation of protein is from about 100:1 to about 500:1 moles CDAP per 100 kDa of the carbohydrate-containing moiety. In another preferred embodiment, the CDAP to carbohydrate-containing moiety ratio for indirect conjugation of protein using a spacer is from 10:1 to 500:1 moles CDAP per 100 kDa of carbohydrate-containing moiety. Depending on the nature of the moieties and the conditions used, different moiety ratios may be optimal.

Unreacted CDAP and reaction by-product such as dimethylaminopyridine can be removed before derivatization or coupling to protein using a suitable purification technique, preferably under acidic conditions, such as dialysis, ultrafiltration, or absorption to suitable bioprocessing beads such as SM4 beads (BioRad). Purified activated polysaccharide can also be prepared by precipitation, e.g., with cold ethanol.

In a preferred embodiment, a carbohydrate-containing moiety that has been activated using CDAP is directly conjugated to the second moiety to produce an immunogenic construct. In another preferred embodiment of the invention, the carbohydrate-containing moiety which has been activated is covalently linked to a suitable bifunctional or heterofunctional reagent. Examples of such functional reagents include ethylene diamine, 1,6-hexane diamine, adipic dihydrazide, cystamine, lysine, glutamic acid, thiol hydrazides, and thiol amines, suitably protected as necessary. See Wong et al., "Chemistry of Protein Conjugate and Crosslinking," *CRC Press* (1991). The second moiety is then covalently linked to the functional reagent, which has already been covalently linked at its other terminus to the carbohydrate-containing moiety.

A preferred pH range for the coupling reaction is from about 7 to about 9, more preferably about 7 to about 8.5. For conjugating a polysaccharide such as dextran, the pH is preferably from about 7.4 to about 8.

A polysaccharide is conjugated to a protein at a ratio in the range of from about 1:1 to about 3:1, e.g., 1:1, using CDAP in one preferred embodiment. For optimal results, high polysaccharide concentrations are avoided. Preferred constructs include tetanus conjugated to a Pneumococcal polysaccharide and tetanus conjugated to *Haemophilus influenzae* PRP. Other preferred conjugates prepared according to the invention include TT-PRP, Pn14-TT, Pn23-TT, malaria-derived peptide-Pn14, DT-Pn14, Pn6-TT, Pn19-TT, and peptide-TT-Pn.

In a preferred embodiment, triethylamine (TEA) is used to facilitate the cyanylation reaction, which may proceed via the formation of an intermediate Von Braun complex. TEA can be replaced by other tertiary amines capable of forming a Von Braun complex. J. Von Braun, *Chem. Ber.*, 33:1438 (1900).

For certain conjugation reactions, glycine, amino ethanol, or other amino-containing reagents may be used to quench the reaction. Such quenching reagents may also be used as one way to modify the net charge of the conjugate.

In another embodiment, the invention relates to vaccines that are made up of an immunogenic construct together with a pharmaceutically acceptable medium or delivery vehicle. Such vaccines will contain an effective therapeutic amount of the immunogenic construct together with a suitable amount of vehicle so as to provide the form for proper administration to the patient. These vaccines may comprise alum or other adjuvants.

Exemplary pharmaceutically acceptable media or vehicles are sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, and the like. Saline is a preferred vehicle when the pharmaceu-

tical composition is administered intravenously. Aqueous dextrose and glycerol solutions can also be employed as liquid vehicles, particularly for injectable solutions. Suitable pharmaceutical vehicles are described in E. W. Martin, *Remington's Pharmaceutical Sciences*, specifically incorporated herein by reference.

The vaccines that may be prepared in accordance with the invention include, but are not limited to, those listed in the chart below:

CHART

Diphtheria vaccine
Pertussis (subunit) vaccine
Tetanus vaccine
H. influenzae type b (polyribose phosphate)
S. pneumoniae, all serotypes
E. coli, endotoxin or J5 antigen (LPS, Lipid A, and Gentabiose
E. coli, O polysaccharides (serotype specific)
Klebsiella, polysaccharides (serotype specific)
S. aureus, types 5 and 8 (serotype specific and common protective antigens)
S. epidermidis, serotype polysaccharide I, II, and III (and common protective antigens)
N. meningitidis, serotype specific or protein antigens
Polio vaccine
Mumps, measles, rubella vaccine
Respiratory syncytial virus
Rabies
Hepatitis A, B, C, and others
Human immunodeficiency virus I and II (GP120, GP41, GP160, p24, others)
Herpes simplex types 1 and 2
CMV (cytomegalovirus)
EBV (Epstein-Barr virus)
Varicella/Zoster
Malaria
Tuberculosis
Candida albicans, other candida
Pneumocystis carinii
Mycoplasma
Influenzae viruses A and B
Adenovirus
Group A streptococcus
Group B streptococcus, serotypes, Ia, Ib, II, and III
Pseudomonas aeroginosa (serotype specific)
Rhinovirus
Parainfluenzae, types 1, 2, and 3
Coronaviruses
Salmonella
Shigella
Rotavirus
Enteroviruses
Chlamydia trachomatis and pneumoniae (TWAR)
Cryptococcus neoformans

The invention also relates to the treatment of a patient by administration of an immunostimulatory amount of the vaccine. The term "patient" refers to any subject for whom the treatment may be beneficial, and includes mammals, especially humans, horses, cows, dogs, and cats, as well as other animals, such as chickens. An "immunostimulatory amount" refers to that amount of vaccine that is able to stimulate the immune response of the patient for the prevention, amelioration, or treatment of diseases. The vaccine of the invention may be administered by any suitable route, but is preferably administered by intravenous, intramuscular, intranasal, or subcutaneous injection.

The invention also relates to a method of preparing an immunotherapeutic agent against infections caused by bacteria, viruses, parasites, fungi, or chemicals by immunizing a patient with the vaccine described above so that the donor produces antibodies directed against the vaccine. Antibodies may be isolated or B cells may be obtained to later fuse with myeloma cells to make monoclonal antibodies. The making of monoclonal antibodies is generally known in the art (see Kohler et al., *Nature*, 256:495 (1975), specifically incorporated herein by reference). As used herein, "immunotherapeutic agent" refers to a composition of antibodies that are directed against specific immunogens for use in passive treatment of patients. A plasma donor is any subject that is injected with a vaccine for the production of antibodies against the immunogens contained in the vaccine.

EXAMPLE 1

Derivatization of a Carbohydrate-Containing Moiety with a Spacer

Materials

CDAP, pyridine, hexane diamine, sodium borate, HEPES, and triethylamine (TEA) were purchased from Aldrich (Milwaukee, Wis.). The carbohydrate-containing moiety, T2000 dextran, with an average molecular weight of 2000 kDa, was obtained from Pharmacia (Piscataway, N.J.).

A stock of CDAP in dry acetonitrile at 100 mg/ml was stored at −20° C. and kept on ice when in use. T2000 dextran was made up at 10.5 mg/ml in saline plus 0.02% azide. Aqueous triethylamine stock was made up at 0.2M and kept on ice during use.

Hexane diamine was made up at 0.5M in 0.1M sodium borate.

Amino group determination was made using trinitrobenzene sulfonate (TNBS) and an extinction coefficient of 11,000 m^{-1} at 366 nm. Franci et al., *J. Imm. Methods.*, 86:155 (1986). Carbohydrate was assayed by the method of M. Monsigny et al., *Anal. Chem.*, 175:525 (1988), using T2000 dextran as the standard.

Control Reactions

The following experiments demonstrate the importance of the components used in the derivatization reaction of the invention. The results show that the amino groups in the final conjugate are covalently linked to the carbohydrate and their presence is not due to artifact or "carryover" of reagent into the final product. Reactions were carried out on ice. For trials performed, omission or substitution of reagents was as indicated in Table 2.

In the procedure using all reagents (line 1 of Table 2), CDAP was added to a vortexed solution of 300 µl dextran (3.1 mg) and returned to the ice bucket. Thirty seconds later, the TEA was added to the vortexed solution. Two minutes after the CDAP was added, 200 µl of the diamine was added and the solution kept on ice for another hour. Samples were dialyzed overnight, filtered with a Millex GV filter, and further desalted on a 1×15 cm P6DG column (BioRad).

As shown in Table 2 below, amino groups were optimally incorporated into dextran in the presence of dextran, CDAP, TEA, and hexane diamine. The data in Table 2 further demonstrate that the amino groups detected are not due to carryover of unconjugated reagents into the final products. Although these results show that TEA is not necessary for derivatization, they show less derivatization when TEA is not present (probably due to a low pH, as later discussed).

TABLE 2

#	Saline	Dextran	100 mg/l CDAP	0.2 M TEA	0.5 M Hexane Diamine	0.1 M Borate	NH_2/ Dextran*
1	0	300 µl	15 µl	15 µl	300 µl	0	64
2	300 µl	0	15 µl	15 µl	300 µl	0	0
3	0	300 µl	0	15 µl	300 µl	—	0
4	0	300 µl	15 µl	0	300 µl	—	2.1
5	0	300 µl	15 µl	15 µl	0	300 µl	0
6	300 µl	0	15 µl	0	0	0	0

*Moles NH_2 per 100 kDa dextran.

Derivatization of T2000 Dextran with Hexane 1,6-Diamine

This experiment demonstrates that CDAP can be used to derivatize carbohydrates to introduce amino groups at both high and low ratios. Dextran T2000 was used as a model carbohydrate. Dextran is a polymer made up of glucose monomers.

The first step in the preparation of many conjugate vaccines is the addition of a spacer (Dick & Beurret, "Glycoconjugates of Bacterial Carbohydrate Antigens," *Conjugate Vaccines*, J. M. Cruse & R. E. Lewis (eds.), Vol. 10, pp. 48–114 (1989)). This series of experiments, summarized in Table 3, emphasizes the ease with which a spacer can be added to polysaccharides.

TABLE 3

#	Dextran (µl)	CDAP (µl)	TEA (µl)	Diamine (µl)	10^{-3} mole CDAP/mole Dextran	NH_2/* Dextran	% Efficiency (NH2/CDAP)**	%*** Derivat'd
1	600	5	5	600	.68	17	50.0	3.1
2	600	10	10	600	1.36	33	48.5	5.9
3	600	15	15	600	2.03	25	24.8	4.6
4	300	15	15	200	4.06	30	16.7	6.1
5	300	30	30	200	8.12	48	11.8	8.2
6	300	60	60	200	16.24	84	4.2	6.2
7	300	120	120	200	32.48	112	6.9	20.4
8	300	15	15	200	4.06	38	18.7	6.9****
9	300	30	30	200	8.12	62	15.3	11.3****
10	300	60	60	200	16.2	35	4.3	6.4****
11	600	15	15	600	2.03	19	18.8	3.5

*Moles NH_2 per 100 kDa of dextran.
**To calculate this value, NH_2/dextran values were divided by mole CDAP/mole dextran values and multiplied by 100%.
***Percent of glucose unit within dextran bound to an NH_2 group.
****Experiment carried out at room temperature.

The experiment was conducted at two temperatures. In the runs summarized in lines 1–7 and 11 of Table 3, all reagents were ice-cold, and in the runs summarized in lines 8–10, the reagents were at room temperature. Procedures and reagents were used as described above for the experiment summarized in Table 2, and reagent amounts added were as indicated in Table 3. In the run represented by line 11, diamine was added in 0.15M HEPES. The reaction was slightly less efficient at lower pH. In another embodiment, hexane diamine was made up in 0.1M borate, pH 9.

Efficiency is defined as the number of moles of spacer groups incorporated per mole of CDAP used, expressed as a percentage. The last column (% derivatized) is the percent of the glucose monomer units of the dextran which have been modified with a spacer.

The results are further illustrated in FIG. 4, which shows the total number of amino groups (e.g., the spacer reagent added) incorporated versus the moles of CDAP added per moles dextran unit. When this data are converted into NH_2 incorporation versus moles CDAP/mole dextran, it is evident that a CDAP:glucose ratio of less than one is sufficient for high levels of NH_2 incorporation. Thus, minimal modification of dextran polysaccharide is necessary for high NH_2-group incorporation.

Furthermore, since an undetermined amount of the active cyanate ester is hydrolyzed without adding a spacer, the CDAP/glucose ratio is an overestimate of the degree of modification of the polymer. Thus, the actual degree of modification is less than the calculated CDAP/glucose ratio.

The degree of incorporation of spacer groups at the lowest reagent dose tested (line 1), 3.1%, is comparable to that used for the synthesis of conjugate vaccines (Chu et al., *Inf. & Imm.*, 40:245 (1983); Dick & Beurret, "Glycoconjugates of Bacterial Carbohydrate Antigens," *Conjugate Vaccines*, J. M. Cruse & R. E. Lewis (eds.), Vol. 10, pp. 48–114 (1989).

The table and figure demonstrate the high efficiency of the CDAP reaction for adding spacer reagents. Further optimization of reaction conditions can increase efficiency. Also illustrated is the very high level of incorporation of spacer groups into polysaccharide which is possible using CDAP. At the highest amount of CDAP added (line 7), approximately 1 in 5 of the glucose units was modified (20%) with a spacer. It is not possible to obtain this degree of incorporation of spacer with cyanogen bromide (Kagedal & Akerstrom, *Acta Chemica Scan.*, 25:1855 (1971)).

During the reactions, there was no evident precipitation of the dextran polysaccharide. In contrast, aggregation and precipitation of the polysaccharide can be a problem with the cyanogen bromide method (Kagedal & Akerstrom, *Acta Chemica Scan.*, 25:1855 (1971)).

These reactions were done in small volumes (<1 ml), thus allowing many trial experiments to be conveniently performed. This is important when optimizing a procedure without wasting valuable carbohydrates and proteins. Thus, from the small volumes of reagents exemplified as well as other information set forth herein, the artisan can readily

practice the invention using larger amounts as desired in any scale-up for commercial use. In contrast, it is difficult to conveniently work with very small amounts of cyanogen bromide due to its poor water solubility, uncertain potency, and toxicity.

Moreover, comparing lines 8–10 of Table 3 with lines 1–7 and 11, it appears that the level of incorporation of amino groups into dextran was approximately the same when the coupling reaction was carried out at 0° C. or room temperature.

Demonstration of Efficiency of Conjugation Reaction Using CDAP and Verification of Conjugation Using Radiolabeled Protein

Since the conjugation reaction using CDAP caused some absorbance at 280 nm, the wavelength normally used to estimate protein concentrations, radiolabeled protein was directly conjugated to dextran. This allowed independent determination of the protein concentration from its specific activity. The yields and recovery of protein were determined.

BSA was lightly radiolabeled with N-hydroxysuccinimide (^{3}H-2,3)-propionate (Amersham), essentially as described by Brunswick et al., *Journal of Immunol.*, 140:3364 (1988). Radiolabeled BSA was dialyzed exhaustively into PBS+ 0.02% azide and subjected to gel filtration chromatography on a S100HR column (Pharmacia) to remove aggregates and concentrated by ultrafiltration using a YM30 filter (Amicon). The BSA concentration was 21 mg/ml, determined from its extinction coefficient at 280 nm (44,000M^{-1}). The specific activity of the stock solution, determined by liquid scintillation counting, was 5.48×10^{12} cpm/mole.

Other reagents were as follows: T2000 dextran (approximately 2000 kDa) (Pharmacia) was dissolved at 10.5 mg/ml in water. CDAP was made up at 100 mg/ml in dry acetonitrile, triethanolamine (TEA) was made up at 0.2M in water. Glycine (pH 5.0) was prepared at 1M in water.

Protocol: Reagents were kept on ice and all reactions were performed on ice. The reaction mixture was vortexed during each addition. Twenty-five µl of CDAP was added to 0.5 ml of dextran (5.25 mg), and 30 seconds later 25 µl TEA was added. After a total of 2.5 minutes, 5.25 mg of radioactive BSA was added. Thirty minutes later, the reaction was quenched by the addition of 100 µl of glycine solution and left overnight at 4° C. An aliquot of 0.6 ml was then filtered using a Spin-X membrane (COSTAR). A comparison of the radioactivity aliquots before and after filtration demonstrated that essentially 100% of the radioactivity was recovered in the filtrate. Five hundred µl of the filtrate was applied to a 1×57 cm S400SF gel filtration column (Pharmacia) which was equilibrated with saline plus 0.02% azide, and run at 0.2 ml/min. Fractions of 0.89 ml were collected and analyzed. Dextran concentrations were determined by the method of Monsigny et al. using absorbance at 480 nm. The radioactivity of a 50-µl aliquot taken from each tube was determined by liquid scintillation counting, and ^{3}H-BSA concentration was calculated using its specific activity. The position of unconjugated BSA in the column elution was determined in an independent column run.

As shown in FIG. 5, a large portion of the BSA, represented by the cpm, is in a high molecular weight form which runs in an identical position as the dextran, represented by OD480. There is a small residual BSA peak representing unconjugated protein. Table 4 contains the purification data.

TABLE 4

Total protein recovered:	3.0 mg
Protein applied to column:	2.9 mg
Recovery:	103%
Protein in high MW form: (tubes 15–23)	>2.0 mg (68%)
Ratio of BSA to DEXTRAN for: 2000 kDa dextran	26

The column did not cleanly separate the dextran-BSA conjugate from the unconjugated protein. This is not unusual since the high molecular weight polymers frequently cause tailing in gel filtration columns. Furthermore, since the T2000 dextran was unfractionated, it contained a spectrum of sizes. To estimate the amount of conjugated BSA in the region where free and bound BSA overlap, a constant ratio of bound BSA to dextran was assumed. Total conjugated BSA, calculated by multiplying the BSA:dextran ratioxthe total molar amount of dextran, was determined as 2.55 mg. This indicates that 87% of the protein was converted to conjugate form.

TABLE 5

Mole CDAP/ mole glucose	mole TEA/ mole CDAP	BSA/dextran	% BSA Conjugated
0.39	1:2	26	87
0.39	2:1	10	34
0.16	1:2	9	28
0.16	5:1	1	3

The results of this BSA-dextran experiment are summarized in Table 5 (line 1) along with three other trials using different amounts of CDAP and TEA (lines 2–4). Both the amount of TEA and the amount of CDAP help get high protein to polysaccharide ratios via direct conjugation. The optimal reagent quantities can easily be determined since the method permits convenient experimentation with small amounts.

It should be emphasized that the direct conjugation reaction does not modify the unconjugated protein, unlike the carbodiimide or heteroligation coupling methods, nor does it use harsh conditions. Thus, one could recover the unconjugated protein for further use. Since many protein antigens are valuable, this is a major advantage of the direct conjugation method.

EXAMPLE 2A

Preparation of PT-Pn14 Conjugates

The purpose of these experiments is to: (1) demonstrate that the transformation of the protein from a low molecular weight form to a high molecular weight form is a result of direct conjugation of the protein to the carbohydrate; (2) determine, under one particular set of conditions, the minimum amount of cyanylating reagent needed to conjugate the protein; and (3) demonstrate that clinically relevant conjugates can be prepared using the method of the invention.

Pertussis toxoid (PT) (from Mass. Public Health Biol. Labs, Boston, Mass.) was dissolved at 0.289 mg/ml in 0.5M NaCl, 0.02M sodium phosphate, pH 8.8. One tenth ml of 0.1M sodium borate, pH 9.1, or 0.75M HEPES, pH 7.5, was added per milliliter of PT. Pneumococcal-type 14 (Pn14) (ATTC lot 83909) was dissolved at 5 mg/ml in 0.15M saline with 0.02% azide. Triethylamine (TEA) was dissolved at 0.2M in water. CDAP was dissolved at 100 mg/ml or 10 mg/ml in acetonitrile (made up and stored at −20° C.). Glycine was made up at 1.0M, pH 5.0. Amino ethanol or other amino reagents can be substituted for glycine/HCl.

Experiment 1

Synthesis of Useful Vaccine Construct with Direct Conjugation: PT-Pn14

Each tube contained 250 μg of Pn14 (50 μl) on ice. At time zero, various amounts of CDAP as indicated in the table were added, and 30 seconds later 25 μl of TEA was added. Two minutes later 1 ml of PT was added. After about 1 hour, 100 μl of glycine solution was added.

Samples were kept at 4° C. overnight. The next day, they were filtered with a Costar 0.45 micron spin filter and run on an HPLC TSK-gel filtration column in 0.2M KCl. Percent HMW is the area of the high molecular weight OD280 conjugate peak versus the OD280 peak indicating unconjugated moiety. It is defined by (percent area void volume peak)/(% area void vol. peak +% area unconjugated moiety peak). The percent areas, obtained from the HPLC runs, were as follows:

TABLE 6

Direct Conjugation Of Pertussis Toxoid to Pn14

#	μmole CDAP/100 kDa Pn14	% HMW
1	1720	100.0
2	520	52.3
3	172	32.8
4	51	31.0
5	17	28.1
6	0 (PT control)	22.0
7	0; no TEA, no PT, (Pn14 control)	—
8	0; no TEA, no Pn14; PT without Borate	11.3

Because the PT control has a HMW of 22%, there may be a small amount of aggregation of the PT caused by the reaction conditions. This set of data also indicates that by varying the CDAP to polysaccharide (Ps) ratio, it is possible to control the ratio of protein to carbohydrate in the final conjugate.

Experiment 2

Conjugation of a Monosaccharide to PT

In this series, 150 μl of a solution of 10 mg/ml glucose, which is monomeric, was substituted for the Pn14 polysaccharide. Conditions similar to Experiment 1 were used except that the PT was made up in HEPES (pH 7.5, M 0.075) buffer instead of borate. Also, 20 μl instead of 25 μl TEA was used. These conditions yielded the following:

#	Condition	% HMW form
1	PT only, no CDAP or TEA	>20%
2	CDAP, TEA (not glucose); + PT	~0
3	Glucose, CDAP, TEA; + PT	~0

Numbers 2 and 3 indicate that CDAP does not polymerize the pertussis toxoid itself and that, therefore, the conversion of the PT to a high molecular weight form is due to its coupling to the high molecular weight polysaccharide and not due to polymerization of the protein. It was evident from the HPLC run that glucose was conjugated to PT because there was a slight increase in the molecular weight of PT.

Experiment 3

Synthesis of Useful Vaccine Construct Via a Spacer: PT-Pn14

Pn14-derivatized with hexane diamine was prepared as follows. Ten μl of CDAP (100 mg/ml in acetonitrile) was added (193 mole CDAP per 100 kDa of polysaccharide). Thirty seconds later 20 μl of TEA (0.2M) was added. After a total of 2.5 minutes had elapsed, 300 μl of 0.5M hexane diamine in 0.1M sodium borate (pH 9.1) was added. After one hour, the solution was dialyzed into water, filtered, and desalted into saline on a P6DG (BioRad) column. The void volume was pooled and concentrated with a Centricon 30 device (Amicon). It was determined to have 33 amino groups per 100 kDa of Pn14 polysaccharide.

Pertussis toxoid was conjugated to the amino-Pn14 using heteroligation chemistry (Brunswick et al.). Fifty μl of 0.75M HEPES buffer (pH 7.5) was added to 0.44 ml of the amino-Pn14. It was iodoacetylated with 10 μl of 0.1M iodoacetyl propionate N-hydroxy-succinimide (SIAP). Pertussis toxoid was thiolated with a 20-fold molar excess of SATA (Calbiochem, La Jolla, Calif.). Each was desalted into saline, mixed, and 1/9 volume of buffer containing 0.75M HEPES, 10 mM EDTA, and 0.5M hydroxylamine was added. The final volume was 1.1 ml. After an overnight incubation, the solution was made 0.2 mM in mercaptoethanol for one hour and then 10 mM in iodoacetamide for 10 minutes, following which it was fractionated on a S400SF gel filtration column (Pharmacia) (see FIG. 6). The void volume peak was pooled and concentrated by pressure filtration on a PM10 membrane (Amicon). Approximately 50% of the pertussis toxoid was recovered in conjugate form. The final conjugate contained 0.7 moles PT per 100 kDa of Pn14 polysaccharide. Protein concentration in the conjugate was determined by the Bradford assay (BioRad) using PT as the standard. Polysaccharide concentration was determined by the method of Monsigny et al. using Pn14 as the standard.

EXAMPLE 2B

Direct Conjugation of a Protein to Pn14 Using CTEA

CTEA offers the advantage of having fewer side reactions than CDAP and leads to purer products, as described in Kohn et al., *Anal Biochem*, 115:375 (1981). Its disadvantage is that it is moisture sensitive, must be weighed out in a closed vessel, and cannot easily be prepared as a stock solution.

One ml of Pneumococcal type 14 polysaccharide (Pn14) (5 mg/ml in saline) is kept at 0° C. CTEA (Available from Aldrich Chemical, Milwaukee, Wis.) is stored under dry nitrogen. Two mg CTEA is weighed out in a closed weighing vessel and added to the cooled, vigorously mixed Pn14. Twenty μl of TEA (0.2M in water) is immediately added while mixing. Sixty seconds later, 5 mg of pertussis toxoid (1.5 mg/ml) is added to the stirred solution. One-half hour later, the reaction is quenched with 200 μl 1M glycine (pH 5.0). After an additional hour, the solution is filtered and passed over an S400SF gel filtration column, equilibrated with saline. The void volume peak is collected and sterile filtered. A 1:1 conjugate is produced.

Addition of Spacer Reagent to Pneumococcal Type 14 Polysaccharide Using CTEA

One ml of Pn14 (5 mg/ml in saline) is kept at 0° C. CTEA (available from Aldrich Chemical, Milwaukee, Wis.) is stored under dry nitrogen. One mg CTEA is weighed out in a closed weighing vessel and added to the cooled, vigorously mixed Pn14. Immediately 20 μl is added to TEA (0.2M in water) while mixing. Sixty seconds later, 300 μl of 0.5M hexane diamine in 0.1M borate (pH 9) is added while mixing. After one hour, the solution is exhaustively dialyzed into saline and sterile filtered. Since a ratio of 187 mole CTEA per 100 kDa Pn14 is used, a conjugate with approximately 18 amines per 100 kDa of Pn14 is produced.

EXAMPLE 3

Direct Conjugation of Pertussis Toxoid to Haemophilus Influenzae Polysaccharide (PRP)

PRP, average MW 350 kDa, was obtained from the Massachusetts Public Health Biological Laboratory. Pertus-

sis toxoid was from the same source. Fifteen μl of CDAP (100 mg/ml) was added to 100 μl (2 mg) of PRP on ice. Thirty seconds later, 30 μl of TEA was added. This represented 319 moles of CDAP per 100 kDa of PRP. After an additional two minutes, 0.75 ml of pertussis toxoid (1.1 mg) was added. Forty minutes later, 200 μl of 1M glycine (pH 5.0) was added to quench the reaction. After one additional hour, the solution was passed over an S400SF gel filtration column equilibrated with saline (see FIG. 7). The void volume was pooled and sterile filtered. The product was determined to have 1.1 PT per 100 kDa of PRP with an overall yield of 68%.

The vaccine prepared by Chu et al., *Inf. & Imm.*, 40:245 (1983), used 377 moles cyanogen bromide per 100 kDa of PRP and had ratios of 1.4 to 2.1 PT per 100 kDa of PRP with yields of less than 50%. Thus, the direct conjugation method of the invention yielded a similar conjugate but with less work, higher yields, and without the use of a toxic reagent.

Since many published protocols for preparing PRP conjugates start with the PRP derivatized with a spacer (Chu et al., Schneerson et al., *J. Exp. Med.*, 152:361 (1980); Dick & Beurret, "Glycoconjugates of Bacterial Carbohydrate Antigens," *Conjugate Vaccines*, J. M. Cruse & R. E. Lewis (eds.), Vol. 10, pp. 48–114 (1989)), CDAP was also used to add a spacer to PRP. The conditions used were as described above but 100 μl of 0.1M hexane diamine in 0.1M borate was added instead of the pertussis toxoid. The product was dialyzed into saline. It was determined to have 102 amino groups per 100 kDa of PRP. Since this is a higher ratio than used in published procedures, even less CDAP could have been used.

EXAMPLE 4

Immunogenic Constructs Useful as Vaccines Prepared Using CDAP Chemistry

Conjugation Using CDAP and a Bifunctional Reagent

In brief, a malaria-derived peptide, p28 (SEQ. ID. NO.:1: Cys Asn Ile Gly Lys Pro Asn Val Gln Asp Asp Gln Asn Lys), from the gamete-specific protein pfs25, was conjugated to tetanus toxoid (TT). P28 has been shown to induce malaria transmission blocking antibodies. CDAP was then used to couple p28-TT to Pneumococcal-type 14 (Pn14) polysaccharide.

FDA-approved tetanus toxoid was dialyzed overnight into HEPES buffer and reacted with a 30-fold molar excess of the iodoacetylating agent (SIAP). After 3 hours, reagents were removed by ultrafiltration using a Macrosep 30 (Filtron Technology) and washed into fresh HEPES, 0.15M, pH 7.5, buffer. Tritium-labeled p28 was added as a solid to the derivatized TT while gently mixing. Following overnight reaction at 4° C., the mixture was treated with 0.2 mM mercaptoethanol to block any remaining active groups and then desalted on a P6DG column equilibrated with HEPES buffer. From the specific activity of the peptide, the product was determined to contain 20 moles p28 peptides/mole of TT. The conjugate was dialyzed into saline and sterile filtered.

Direct Conjugation Using CDAP

Pn14 (obtained from American Tissue Type Collection, ATTC) has a high molecular weight (c.a. 10^6 daltons). P28-TT was directly conjugated to Pn14 as follows. CDAP (10 μl from a 100 mg/ml stock solution in acetonitrile) was added to Pn14 (1.1 mg in 150 μl saline). Thirty seconds later, 20 μl of triethylamine (0.2M) was added. Two minutes later, 0.55 mg in 0.8 ml saline) of p28-TT was added, and one hour later, the reaction was quenched for another hour with 200 μl 1.0M glycine (pH 5). The conjugate was then passed over an S400SF gel filtration column equilibrated with saline and the void volume containing the conjugate was pooled. FIG. 9 indicates that virtually all of the p28-TT was found in the void volume in conjugated form.

Immunoreactivity of Immunogenic Constructs

Groups of 5 DBA/2 mice were immunized with i.v. with 10 μg p28-TT or (p28-TT)-Pn14 conjugate in saline, bled three weeks later, and the sera assayed by ELISA (enzyme-linked immunoabsorbent assay) for reactivity against recombinant pfs25 protein. Peptide p28 is derived from pfs25. Another set of mice was immunized with the same antigens precipitated with the adjuvant, alum (Imject, Pierce Chemical Co., Rockford, Ill.).

Consistent with the related applications, Table 7 shows that only the high molecular weight conjugate elicited good anti-protein titers.

TABLE 7

	Anti-pfs25 IgG1 Titers	
Antigen	i.v. (saline)	s.c. (alum)
(p28-TT)-Pn14	36	346
p28-TT	<10	<10

This demonstrates that the CDAP method can be used to prepare useful vaccine constructs. It also illustrates the ease with which useful conjugates can be prepared.

EXAMPLE 5

Biologically Active Multivalent Protein Constructs Prepared Using CDAP

To demonstrate that conjugates prepared using CDAP to directly couple proteins to polysaccharides could yield a multivalent product (which as set forth in the related applications has enhanced immunogenicity) and that the process could be gentle enough to preserve biological activity, various conjugates of a monoclonal antibody with dextran were prepared. These experiments used monoclonal antibody Hδ^a/1 with an anti-IgD antibody which crosslinks membrane IgD on B lymphocytes and induces proliferation (Brunswick et al., *Journal of Immunol.*, 140:3364 (1988)). As described by Brunswick et al., conjugation of multiple copies of Hδ^a/1 to a high molecular weight polymer such as 2000 kDa dextran (Hδ^a/1-AECM dextran) induced B-cell proliferation at 1000-fold lower concentrations and induced higher levels of proliferation than unconjugated Hδ^a/1. In Brunswick et al., a simple, straightforward but multistep, multi-day procedure was required to prepare the conjugate. Aminoethyl carboxymethyl dextran (AECM dextran) was prepared first as described in Brunswick et al. and then heteroligation chemistry was used to couple the Hδ^a/1 to the carbohydrate.

Hδ^a/1-dextran was prepared by both direct conjugation using CDAP and indirect conjugation using a spacer and CDAP as follows.

Direct conjugation: To a vortexed solution of 3.2 mg of T2000 dextran (Pharmacia) in 0.3 ml saline, 15 μl of CDAP was added (from a 100 mg/ml stock in acetonitrile). Thirty seconds later, 15 μl of 0.2M TEA was added while vortexing. After an additional 2 minutes, 6 mg Hδ^a/1 (in 362 μl 10.05M sodium borate and 0.075M NaCl) was added while gently vortexing. After 15 minutes, the reaction mixture was quenched by the addition of 100 μl of 1.0M glycine, pH 5.0, and passed over an S400SF gel filtration column (1×59 cm) equilibrated with saline. The column elution is shown in FIG. 9. The void volume peak was pooled and sterilized with a Millex GV filter. The product is called Hδ^a/1-(CDAP)-dextran. This procedure took approximately 3 hours.

Spacer: Dextran was activated with CDAP as above (31.5 mg T2000 dextran in 3 ml saline and 25 µl CDAP followed by 25 µl TEA. 1 mole CDAP/0.06 mole of glucose monomers). Three ml of 0.5M 1,6-diaminohexane in 0.1M sodium borate was added. The solution was exhaustively dialyzed into water and then fractionated on an S400HR gel filtration column. The void volume was pooled and concentrated. This amino-dextran was determined to have 147 amino groups per 2000 kDa dextran. The product is called NH_2-(CDAP)-dextran. Including dialysis, this was a two-day procedure. In contrast, AECM-dextran usually takes about one week to prepare using the Brunswick et al. method.

Hδ°/1 was conjugated to AECM-dextran and NH_2-(CDAP)-dextran using the heteroligation techniques described in Brunswick et al. The conjugates are called Hδ°/1-AECM-dextran and Hδ°/1-NH_2-(CDAP)-dextran, respectively. Conjugation using ACEM-dextran was a two-day procedure.

B-cell proliferation assays, using 10,000 cells/well, were performed as described by Brunswick et al. Table 8 provides the results of those assays, specifically indicating incorporation of tritiated thymidine into B cells as counts per min./well.

TABLE 8

	Hδ°/1 Concentration (µg/ml)		
Mitogen	1	0.1	0.01
Hδ°/1-AECM-dextran (preparation 1)	16,045	25,774	25,850
Hδ°/1-AECM-dextran (preparation 2)	21,685	29,280	34,969
Hδ°/1-(CDAP)-dextran	16,497	23,654	19,779
Hδ°/1-NH_2-(CDAP)-dextran	19,353	28,343	25,879
Medium (control)	760	725	760

As reported in Brunswick et al., Hδ°/1 alone causes no incorporation at these concentrations. Maximum incorporation at 10–100 µg/ml Hδ°/1 is approximately 3000 cpm.

This data indicate that the conjugates prepared using CDAP, with and without a spacer, are essentially equivalent to Hδ°/1-AECM dextran in their abilities to induce proliferation. Since only multivalent antibody induces high levels of proliferation at low doses, all the conjugates must be multivalent. Thus, direct conjugation with CDAP did not affect the biological activity of the antibody. The direct conjugation procedure was markedly faster to prepare than conjugates prepared with a spacer. Further, adding the spacer and conjugating using CDAP was much faster than preparing AECM dextran.

Thus, this experiment illustrates (1) the high yield of a multivalent construct using CDAP and (2) the ease and speed of preparation of conjugates, especially direct conjugates. Conjugation using CDAP and a bifunctional reagent took under 48 hours and direct conjugation took less than three hours.

EXAMPLE 6

Unless indicated otherwise, the protocol in these experiments was generally as follows. Triethylamine (TEA), acetonitrile, sulfuric acid (H_2SO_4), resorcinol, hexane diamine, sodium borate, and HEPES were obtained form Aldrich and were of reagent grade or better. N-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) was purchased from Sigma or from Research Organics (Cleveland, Ohio). Trinitrobenzene sulfonic acid (TNBS) was obtained from Kodak Chemicals. Millex filters were obtained from Millipore Corp.

Dextran T2000 was obtained from Pharmacia. Pneumococcal type 14 polysaccharide was obtained from the ATTC (Rockville, Md.). Amino ethyl carbamyl dextran was prepared as described by Brunswick et al. Monomeric BSA (bovine serum albumin) was prepared from low endotoxin Cohen fraction V BSA (Sigma catalogue #A9306) by gel filtration on a 2.6 cm×97 cm S100HR column (Pharmacia), equilibrated with saline plus azide. The product was shown by analytical HPLC to have less than 0.5% dimer and less than 0.1% material of higher molecular weight mass. The BSA was periodically checked by HPLC to confirm its monomeric status. An extinction coefficient of 44,000M^{-1} was used for BSA.

Polysaccharide was activated with CDAP as follows. CDAP was made up at 100 mg/ml in acetonitrile and stored at −20° C. for up to one month. CDAP was slowly pipetted into a vortexed solution of polysaccharide in water, and thirty seconds later, a volume of 0.2M TEA equal to the volume of CDAP used was added. At 2.5 minutes, a one-fifth volume of 0.5M hexane diamine in 0.1M sodium borate (pH 9.3) was added. The reaction proceeded overnight at 4° C. The reaction product was desalted on a P6DG or a P6 cartridge (BioRad), equilibrated with saline, and then further dialyzed into saline. Some samples were concentrated using a Centricon 30 device (Amicon) and desalted again to confirm the removal of free diamine. Variations of this general procedure are indicated below. The extent of derivatization with hexane diamine was determined using a TNBS assay for primary amines. Absorbance was measured at 366 nm, using an extinction coefficient of 11,000M^{-1} (Franci et al.). CDAP-activated dextran, derivatized using ethanolamine instead of diamine, was found to be TNBS negative in this assay. Polysaccharide concentrations were determined as described by Monsigny et al. Results are expressed as moles of amine per 100 kDa of polysaccharide unless indicated otherwise.

Protein conjugation to amino-dextran via a thio-ether linkage was performed as described by Lees et al., *Vaccine*, 12(3):1160, 1994. Protein was conjugated directly to polysaccharide by activating the polymer with CDAP as described above for derivatization with amines. Protein (10 mg/ml in 0.15M HEPES, pH 7.5) was rapidly added to a gently vortexed solution at 2½ minutes after the CDAP was introduced. Reactions were quenched with approximately ⅕ volume 0.5M ethanolamine in 0.75M HEPES, pH 7.5, for at least one hour before gel filtration on a S300HR or S400HR column (Pharmacia), equilibrated with saline. The peak tube from the void volume was assayed for protein with the Bradford method (BioRad reagent) using BSA as the standard. Polysaccharide concentrations were determined by the method of Monsigny et al., using dextran as the standard. The results, which are discussed below, are expressed as mg of protein per mg of polysaccharide unless indicated otherwise.

Activation of Polysaccharides Using CDAP

Experiments were performed to determine whether CDAP activation of polysaccharides can be used to prepare conjugate vaccines under conditions that are more rapid, more gentle, more convenient, and safer than previously reported methods. As a prototype polysaccharide, high molecular weight dextran (T2000 dextran, Pharmacia) was activated with CDAP under a variety of experimental conditions.

From a 100 mg/ml stock solution, a volume of CDAP was slowly pipetted into a solution of T2000 dextran in water (1.6 mg/ml as shown in FIG. **4**, or 10 mg/ml as shown in FIG. **10**). At 30 seconds, a volume of 0.2M TEA equal to the volume of CDAP was added, and 120 seconds later, a large excess of hexane diamine in sodium borate (pH 9.3) was quickly added. After desalting on a P6DG column followed by exhaustive dialysis to remove unconjugated reagents,

high levels of polysaccharides were found (see FIGS. 4 and 10). Following this same procedure but in the respective absence of CDAP, the dextran, or the diamine, no amines were detectable using the TNBS assay. Furthermore, CDAP-activated dextran reacted with a monoamine (ethanolamine), instead of the hexane diamine, was TNBS negative. To further ensure that all low molecular weight material had been removed, the derivatized polysaccharide was concentrated by ultrafiltration and desalted a second time on a P6DG column. The amine ratio was unchanged after this procedure.

The degree of derivatization was dependent on the amount of CDAP—increases in the CDAP-to-dextran ratio led to increases in the absolute number of amino groups substituted onto the polysaccharide as shown in FIGS. 4 and 10. The extent of derivatization was dependent on the polysaccharide concentration for the same molar CDAP-to-dextran ratio. Thus, at 1.6 mg/ml dextran, efficiencies ranged from 0.7 to 2.4 percent based on moles of amines substituted per mole of CDAP, while at 10 mg/ml dextran, as much as 0.2 mole of amines were substituted per mole of CDAP (20% efficiency).

In order to improve the efficiency of this bimolecular reaction, the polysaccharide concentration was increased from 1 to 50 mg/ml, using a fixed amount of CDAP (see FIG. 11). At the highest polysaccharide concentration used, more than 0.4 mole of amine was added for every mole of CDAP used. In contrast to the high level of substitution attained with CDAP activation, CNBr activation usually yields maximum efficiencies of about 1 to 2%.

In the absence of TEA, derivatization with diamines was markedly reduced. To determine whether the presence of a tertiary amine such as TEA is essential for activating a soluble polysaccharide with CDAP, the efficiency of activation using TEA was compared with that using inorganic buffer or NaOH.

One hundred µl of a CDAP solution (100 mg/ml in acetonitrile) was slowly added to a stirred solution of 2 ml of T2000 dextran (10 mg/ml in water) at room temperature. After thirty seconds, 1N NaOH was slowly added to maintain the pH at about 9. After 1½ minutes, 1 ml of BSA, 20 mg/ml in 0.5M HEPES, pH 8.0, was added. After the reaction was allowed to proceed for eighteen hours at 4° C., it was quenched by adding 100 µl of 0.5M ethanolamine in 0.75M HEPES, pH 7.5. For analysis, 300 µl of the product was gel-filtered on a 1 cm×50 cm S400HR column equilibrated with saline and azide. The void volume peak tube was assayed for protein using the BioRad assay and for polysaccharide using the resorcinol assay, and was found to have 0.45 mg of BSA per mg of dextran.

As shown in Table 9 below, derivatization resulted with a variety of buffers. Indeed, careful addition of 1N NaOH was used to raise the pH to about nine yielded good levels of substitution.

TABLE 9

Derivatization of dextran with hexane diamine using various buffers (desalted, dialyzed, concentrated, and desalted)

Buffer	NH_2/100 kDa dex
TEA (0.2 M)	29
Borate (pH 8.8)	40
Carbonate	20
NaOH	36

With dextran, there were no significant differences in the levels of derivatization over a pH range of from 8 to 10, although other polysaccharides have been found to be more dependent on the activation pH (see below). As noted above, if TEA is omitted and the pH is not raised, the dextran is still activated but it is derivatized to a much lower degree. Thus, CDAP activation or coupling does not depend on the presence of TEA or a buffer—any appropriate means may be used to raise the pH so that the reaction mixture is sufficiently alkaline.

Table 10 shows the reaction kinetics of activation using CDAP. In the experiment, 100 µl CDAP (100 mg/ml acetonitrile) was added to 1 ml dextran (20 mg/ml) at 30 seconds, 1 ml of 0.1M sodium borate, pH 8.8, was added, and after two minutes, 0.5 ml hexane diamine in 0.75M HEPES was added. Aliquots were desalted at the indicated times on a P6 cartridge equilibrated with saline, and then exhaustively dialyzed into saline before analysis. At high concentrations of polysaccharide and CDAP, the solutions gelled. Thus, it is more convenient to work with 10 to 20 mg/ml polysaccharide solutions.

TABLE 10

Kinetics of Reaction of CDAP-Activated Dextran with Hexane Diamine

Reaction time	NH_2/100 kDa dex
15 mim.	42
1 hr.	46
3 hr.	47
24 hr.	48

As shown in Table 10, the derivatization reaction was rapid and essentially complete within 15 minutes. No increase in the degree of derivatization was noted at 3 or 24 hours.

To test reproducibility, Pneumococcal polysaccharide type 14 (Pn14) was activated with CDAP and derivatized with hexane diamine. To a stirred solution of 1 ml of Pn14 (10 mg/ml in water) was added 30 µl of CDAP (100 mg/ml in acetonitrile) (0.3 mg CDAP/mg Pn14). After thirty seconds, 30 µl of TEA (0.2M in water) was added. At two minutes, 0.5 ml of hexane diamine (0.5M in 0.75M HEPES, pH 7.6) was added. At 1½ hours, the product was desalted with P6 cartridge, concentrated by ultrafiltration, and again desalted, and then assayed for amines with TNBS and for Pn14 with resorcinol/sulfuric acid. As shown in Table 11, efficiencies of 13–15%, based on moles of amines detected per mole of CDAP used, were obtained in three experiments performed over a one-year period.

TABLE 11

Experiment	NH_2/100 kDa dex	Efficiency (mole NH_2/mole CDAP)
A	17.9	14.1%
B	19.8	15.5%
C	17.3	13.6%

The results tabulated above indicate stability of the CDAP reagent in the freezer, reproducibility, and high efficiency. In comparison, CNBr solution is not stable, and the CNBr-activation procedure is difficult to reproduce and has an efficiency of about 2%.

Direct Conjugation of Protein to CDAP-Activated Ps

As with derivatization of amines, the extent of protein conjugation to the polysaccharide was dependent on the amount of CDAP used to activate the polysaccharide. As shown in FIG. 12, at a concentration of 10 mg/ml dextran, the CDAP:dextran ratio linearly increased with the BSA-:dextran ratio of the product. Similar ratios of BSA:dextran could also be observed at even lower CDAP:dextran ratios if the protein and/or polysaccharide concentrations were increased.

Control reactions performed in the absence of dextran and analyzed by gel filtration indicated that the CDAP by itself did not aggregate or polymerize the BSA (protein). A CDAP-treated sample (0.5 ml water+25 µl CDAP @100 mg/ml in acetonitrile+50 µl 0.2M TEA+0.5 ml BSA @10 mg/ml in 0.5M HEPES, pH 8.0) and a control sample (0.575 ml water+0.5 ml BSA (monomeric) @10 mg/ml in 0.5M HEPES, pH 8.0) were prepared. The samples were allowed to react overnight and were quenched with 100 µl of 0.5M ethanolamine in HEPES. After quenching for one hour, the samples were run on a S400 1 cm×50 cm column in saline and azide at 0.75 ml/minute. The OD280 over the column was summed and divided into the sum of the OD280 over the tubes preceding the BSA peak. The CDAP-treated sample showed 0.6% polymeric BSA, and the control sample showed 0.7% polymeric BSA. Thus, the high molecular weight protein is not due to self-polymerization or aggregation.

Moreover, under normal conditions, CDAP does not crosslink the polysaccharide. This was confirmed by the following HPLC experiment where 70 kDa of dextran was activated and then reacted with ethanolamine and run on a gel filtration column. Specifically, 2.5 mg T70 dextran (10 mg/ml) was combined with 20 µl of CDAP (100 mg/ml). At thirty seconds, 20 or 60 µl of 0.2M TEA was added, and at two minutes 100 µl of 0.5M ethanolamine in 0.75M HEPES, pH 7.6, was added. After one hour, samples were run on a G4000 PWXL (Tosohaas) or an SEC3000 (Beckman) in 0.2M NaCl and detected by refractive index (void volume for each column was about 5 minutes, eluting salt at about 10 minutes). No evidence of a shift to higher molecular weight was observed.

As the following comparative experiment shows, extreme conditions should be avoided to prevent the CDAP from crosslinking the polysaccharide. One ml of T2000 dextran (100 mg/ml water) was combined with 176 µl of CDAP (100 mg/ml). After thirty seconds, 176 µl of 0.2M TEA was added, which yielded a gel in less than two minutes.

To determine the optimum activation time and to examine stability of the CDAP-activated polysaccharide, protein (BSA) was added 5–300 seconds after the addition of the CDAP and TEA, and the BSA:dextran ratio of the product was determined. The results shown in FIG. **13** suggest that the optimal activation time is about 2 minutes and that the activated polysaccharide is stable over this time period. If the protein is added at one hour, the reaction yield declines by about one third.

Aqueous mixtures of CDAP and polysaccharides were found to be stable, as reflected in FIG. **14**. Sixty µl of CDAP (100 mg/ml) was added to 1 ml water, and 100-µl aliquots of this CDAP solution were combined with 100 µl of polysaccharide (dextran, 20 mg/ml) at various times over a period of 10–300 seconds as shown in FIG. **14**, followed 30 seconds later by combination with 15 µl of a TEA solution (0.2M). Two minutes after being combined with the TEA, 100 µl of BSA (30 mg/ml) was added. The reaction was quenched at 48 hours.

No significant differences were found in the final protein-to-polysaccharide ratios over the entire range of addition times. The results shown in FIG. **14** are consistent with the stability of CDAP in acidic solutions and the observation that solutions of CDAP in water become acidic. Thus, water can be substituted for the organic solvent if the reagent solution is to be used the same day. Alternatively, CDAP can be added as a solid to the solution of polysaccharide. In working with small amounts of CDAP, it has been found more convenient to work with solutions than to work with the solid reagent. Furthermore, whereas the rapid addition of an acetonitrile solution of CDAP will sometimes precipitate the polysaccharide, precipitation can be avoided if an aqueous solution of CDAP is used. Aqueous stock solutions of CDAP can be prepared at concentrations up to 75 mg/ml.

FIG. **15** shows that protein conjugation to the polysaccharide was relatively rapid, and within three hours 80% of the maximum conjugation had been attained. Even more rapid coupling could be achieved by increasing the protein concentration, the polysaccharide concentration, and/or the CDAP concentration.

As indicated in FIG. **16**, the pH of the reaction solution during the polysaccharide activation is another important parameter in polysaccharide activation with CDAP. As the pH during the activation step was increased from 7.0 to 8.3, there was an increase in polysaccharide activation as reflected by a marked increase in coupling efficiency. The BSA:dextran ratio of the conjugate increased 4-fold as the pH increased from 7.0 to 8.3. At a pH higher than 8.3, there was little or no increase in the ratio. The pH dependence of CDAP activation explains the low level of derivatization that was previously observed in the absence of TEA, since the pH of a CDAP solution in water is initially near neutral and becomes more acidic.

As was noted earlier with respect to the derivatization of polysaccharides with amines, a tertiary amine buffer is not necessary during activation of the polysaccharide for the direct conjugation of proteins. Thus, direct conjugation of protein to polysaccharides may be done, e.g., using a pH stat or automatic titrator to raise the pH during the activation step. This could be advantageous in preparing vaccine conjugates.

FIG. **17** illustrates that the pH of the reaction solution during the coupling of the protein to the activated polysaccharide is an important parameter in the direct conjugation of protein with CDAP. In the experiment for which results are reported in FIG. **17**, several buffers were tested over a wide range of pH values and at a low protein-to-polysaccharide ratio. The protocol was as follows.

To four ml of T2000 dextran (10 mg/ml in water) was added 133 µl of a CDAP solution (100 mg/ml in acetonitrile, freshly prepared) (0.33 mg CDAP/mg dex). After 30 seconds, 266 µl of TEA (from a 0.2M stock) was added, and the pH reached a maximum of 9.6. After 2½ minutes, the pH was adjusted to 5.0 using 60 µl of 1M NaAc (sodium acetate). Four hundred µl of activated dextran was transferred to tubes containing 200 µl of BSA (15 mg/ml) (0.8 mg BSA/mg dex) and 100 µl of a buffer (1M NaAc, pH 4.7, 5.7; 0.5M HEPES, pH 6.94, 7.43, 8.15; 0.1M $NaPO_4$, pH 8.0, 8.67; 50 mM sodium borate, pH 9.0, 9.6) (not controlled for ionic strength). One hour after transfer, 350 µl of the solution of a tube were combined with 100 µl of freshly prepared 0.5M ethanolamine in 0.75M HEPES (pH 7.5). Twenty hours later, 100 µl of ethanolamine were added to the remaining solution. The reaction was quenched for at least two hours and the product run on S300HR or S400HR columns equilibrated with saline plus azide. The peak void volume tube was assayed for BSA using the BioRad assay and for polysaccharide using the resorcinol assay.

As shown in FIG. **17**, most of the protein was coupled to the polysaccharide at a pH as low as 7.4, a substantial amount was coupled at a pH as low as 6.9, and a small but significant amount was coupled even at a pH as low as 5.7. For the conditions of this experiment, a pH of about 8 appeared to be optimal. Although the results show that the pH of the coupling step is important, they show that coupling can be done over a wide pH range. Since the coupling reaction is so inefficient at a pH of 5, however, quenching should be done at about a pH of 7 to 8.

Increased amounts of coupling can be obtained even at low pH by increasing the protein-to-polysaccharide ratio, the polysaccharide concentration, and/or the amount of CDAP used. For example, by using more reagent or more protein, higher yields can be obtained even at a pH of 7. Thus, direct protein coupling can be achieved at a near-neutral pH using CDAP to activate the polysaccharide.

FIG. 17 indicates that phosphate is also inhibitory to the coupling reaction, which may be due to ionic interactions or to the slight nucleophilic character of the phosphate. Increasing the amount of CDAP and the pH during the coupling, however, will increase the conjugation ratio/yield. If phosphate is present during the CDAP activation, addition of the diamine is inhibited.

Phosphates of PRP and Pn6 may cause inhibition, as shown by the following experiment. Twenty µl of CDAP (100 mg/ml in acetonitrile) was added to a vortexed solution of 2 mg Pn6 (Pneumococcal type 6, a polyribitol phosphate polysaccharide) (10 mg/ml in water). Thirty seconds later, buffer (100 µl of 0.1M sodium borate or 40 µl of 0.2M TEA) was added. At two minutes, 100 µl of BSA (20 mg/ml) in 0.5M HEPES, pH 8, was added. After incubating overnight at 4° C., the reaction was quenched with 100 µl of 0.5M ethanolamine in 0.75M HEPES, pH 7.5, followed by gel filtration on an S400HR column (Pharmacia) equilibrated with saline and 0.02% azide. The peak void volume tube was assayed for protein and polysaccharide. For comparative purposes, in trial 4 dextran was derivatized in the same manner. The results are reported in Table 12 below.

TABLE 12

Trial	Ps	Buffer	BSA/Ps (mg/mg)
1	Pn6	0.2 M TEA	0.06
2	Pn6	0.1 M sodium borate (pH 8.8)	0.16
3	Pn6	0.1 M sodium borate (pH 10)	0.31
4	dex	0.1 M sodium borate (pH 8.8)	0.77

For Pn6 with the TEA buffer (trial 1), the yield was very low. As the pH was increased with sodium borate (trials 2 and 3), the yield increased. The same conditions give much higher yields for dextran (see, e.g., trial 4). Thus, phosphate-based polysaccharides such as Pn6 require adjustment in the pH and/or CDAP ratio to prepare conjugates in good yields.

The next experiment shows that the isourea bond formed by CDAP activation is stable and robust. In this experiment, ε-TNP-lysine was coupled to dextran via CDAP. Samples 1–5 were made up as follows:

1: 400 µl TNP/CDAP/dex+100 µl saline (control)

2: 400 µl TNP/CDAP/dex+100 µl 2M NaCl

3: 400 µl TNP/CDAP/dex+100 µl 9M GuHCl

4: 400 µl TNP/CDAP/dex+100 µl saline (reacted in incubator @37° C.)

5: 400 µl TNP/CDAP/dex+100 µl saline (control) The samples were allowed to react overnight in the dark, except example 4, which was reacted as indicated. The samples were then desalted on a P6 cartridge in 10 mM sodium borate at 1.0 ml/minute. The fractions were read at OD366 and the peak tube of the void fractions was assayed. The results are provided in Table 13 below.

TABLE 13

Sample	TNP (µM)	Dex (µM)	TNP/100 kDa dex
1	96	9.7	10
2	134	12	11
3	127	11	12
4	137	13	11
5	107	10	11

For each sample the TNP:dextran ratio was unchanged, indicating that the isourea bond was stable to the test conditions.

Biological Activity of Conjugates

To determine whether CDAP activation of the polysaccharide had any detrimental effect on its ability to induce antibody responses, its biological activity in vitro was tested. BSA was either directly conjugated to CDAP-activated Pneumococcal polysaccharide type 14 or coupled to Pneumococcal polysaccharide type 14 derivatized with hexane diamine followed by iodoacetylation and reaction with thiolated protein (Lees et al.). Each conjugate had a ratio of mg BSA/mg Pn14. Inbred DBA/2 mice were immunized subcutaneously with 50 µg of BSA, either free or as a polysaccharide conjugate, in the absence of adjuvants. Sera were collected 14 and 28 days later, and anti-BSA and anti-Pn14 antibody titers determined by ELISA.

Neither unconjugated BSA nor unconjugated Pn14 stimulated a detectable primary response. In contrast, the BSA-Pn14 conjugates stimulated significant antibody responses to both the protein and polysaccharide components, regardless of whether the protein was coupled by indirect conjugation using a spacer or by direct conjugation. Mice immunized with BSA-dextran prepared using a spacer or direct coupling to CDAP-activated dextran gave titers comparable to those obtained when conjugates were prepared using other chemical methods. Moreover, TT-PRP conjugates prepared using CDAP activation have shown in rats immunized with the conjugates anti-PRP responses comparable to those shown in rats immunized with TT-PRP conjugates prepared using CNBr activation. Furthermore, tetanus conjugated directly to CDAP-activated Pn14 had high anti-tetanus and anti-Pn14 antibody responses; opsonic assays indicated that these antibodies were protective.

Summary

The method of the invention utilizing CDAP represents a reproducible approach that can be used to activate various clinically relevant polysaccharides, some of which are sensitive to a high pH. Activation is rapid, so the time is spent at a high pH is minimized. The method produces highly immunogenic protein-polysaccharide conjugates, which can stimulate in mice humoral antibody to both the protein and polysaccharide components even in the absence of adjuvant.

The variables which have been found to profoundly influence the extent of polysaccharide activation are the concentrations of CDAP and polysaccharide, and the pH. A preferred pH for conjugating is about 7 to about 9, more preferably about 7.4 to about 8.0, which is a range at which most polysaccharides are stable. Other pH ranges, e.g., a range of from about 7 to about 10, may be more suitable for other polysaccharides.

By manipulating the polysaccharide and/or CDAP concentration, the efficiency of derivatization can be increased to 50%, as compared to the 1–2% found with CNBr. Furthermore, a product with greater than 50 NH_2 groups per 100 kDa of polysaccharide can be achieved under the preferred conditions. The method of the invention does not depend on the presence of tertiary amines, as has been described by previous investigators experimenting with CDAP. The activation of the polysaccharide is rapid. Similarly, protein conjugation to activated polysaccharide is rapid.

The invention offers the advantages of reproducibility, rapid reactivity, and perhaps most notably, the ability to easily manipulate protein:polysaccharide ratios. For example, conjugates with various protein-to-polysaccharide ratios can be achieved by altering the concentration of CDAP and/or the polysaccharide concentration and/or the protein concentration. This may provide an approach to studying not only the role of protein:polysaccharide ratio in influencing the magnitude of the antibody response to the conjugate, but also the role of the three-dimensional structure at a given protein:polysaccharide ratio.

The immunogenicity of the protein-polysaccharide conjugates prepared using CDAP is significantly greater than

the response demonstrated by either of the unconjugated components. Furthermore, the antibody that is produced is reactive with the unconjugated protein, and the response can be boosted using the unconjugated protein as well as the conjugated protein. This suggests that any chemical alteration of the protein during conjugation has no detrimental effect on its ability to stimulate antibodies with reactivity to the native protein, nor on its ability to stimulate B cells with reactivity to the unconjugated protein.

Additionally, CDAP-activated polysaccharides can be used in preparation for conjugation of anti-Ig antibodies. Anti-Ig-dextran conjugates induce about 100- to 1000-fold greater activation of B cells as compared to unconjugated Ig. Anti-Ig-dextran conjugates prepared using direct conjugation to CDAP-activated dextran are as effective B-cell stimulatory reagents as the conjugates prepared using other heteroligation coupling to AECM dextran.

CDAP is useful for preparing a variety of immunological reagents, such as biotinylated polysaccharides for ELISA and ELISA spot antigens and TNP-polysaccharides (e.g., TNP-dex, TNP Ficoll) for model Ti-2 antigens.

Thus, the inventive method, which employs CDAP to produce immunogenic constructs such as polysaccharide-based conjugates, offers many advantages to the currently available technology for preparing immunogenic constructs. It will be apparent to those skilled in the art that various modifications in the methods and embodiments of the present invention can be made without departing from the scope or spirit of the invention. Thus, the invention should not be construed to be limited by the description and drawings, but by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 1

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Asn Ile Gly Lys Pro Asn Val Gln Asp Asp Gln Asn Lys
1 5 10

What is claimed is:

1. In a method for preparing a vaccine comprising an immunogenic construct and a pharmaceutically acceptable carrier, the improvement comprising producing the immunogenic construct by a process comprising:

(a) activating a viral, fungal or bacterial polysaccharide with an organic cyanylating reagent selected from the group consisting of 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate, N-cyanotriethyl-ammonium tetrafluoroborate, and p-nitrophenylcyanate, to form an activated carbohydrate; and

(b) coupling said activated carbohydrate directly or indirectly to a protein to form the immunogenic construct capable of stimulating an immune response.

2. A method according to claim 1, wherein said organic cyanylating reagent is 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate.

3. A method according to claim 2, wherein said polysaccharide and said protein are soluble in water.

4. A method according to claim 3, wherein said activating is carried out at a pH of from 8 to 10, and said coupling is carried out at a pH of from 7 to 9.

5. A method according to claim 2, wherein said activating is carried out in the presence of triethyl amine.

6. A method according to claim 1, wherein said coupling is done indirectly by covalently joining the polysaccharide to a bifunctional or heterofunctional spacer reagent, and covalently joining the protein to the spacer reagent.

7. A method according to claim 6, wherein said spacer reagent is selected from the group consisting of ethylene diamine, 1,6-hexane diamine, adipic dihydrazide, cystamine, glycine, and lysine.

8. A method according to claim 1, wherein the polysaccharide is selected from the group consisting of dextran, Pneumococcal polysaccharide, *Haemophilus influenzae* polysaccharide, Group A streptococcus polysaccharide, Group B streptococcus polysaccharide, and *N. meningitidis* polysaccharide.

9. A method according to claim 1, wherein the polysaccharide is a water-soluble viral or bacterial polysaccharide.

10. A method according to claim 1, wherein the protein is a water-soluble protein.

11. A method according to claim 1, wherein the protein is selected from the group consisting of bovine serum albumin, pertussis toxoid, tetanus toxoid, malaria-derived peptide, an antibody, a toxoid, and a lipoprotein.

12. A method according to claim 1, wherein the immunogenic construct is a conjugate selected from the group consisting of PT-Pn, PT-PRP, TT-Pn, antibody-dextran, and peptide-TT-Pn.

13. A method for producing an immune response in a patient comprising:

(a) preparing a vaccine comprising an immunogenic construct capable of stimulating an immune response and a pharmaceutically acceptable carrier, wherein the immunogenic construct is produced by; (i) activating a viral, fungal or bacterial polysaccharide with an organic cyanylating reagent selected from the group consisting of 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate, N-cyanotriethyl-ammonium tetrafluoroborate, and p-nitrophenylcyanate, to form an activated carbohydrate, and (ii) covalently joining said

activated carbohydrate to a protein to form the immunogenic construct; and

(b) administering the vaccine to said patient.

14. A method according to claim **13**, wherein said organic cyanylating reagent is 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate.

15. A method according to claim **14**, wherein said activating is carried out in the presence of triethyl amine.

16. A method according to claim **14**, wherein the protein is a water-soluble protein.

17. A method according to claim **14**, wherein the polysaccharide is selected from the group consisting of dextran, Pneumococcal polysaccharide, *Haemophilus influenzae* polysaccharide, Group A streptococcus polysaccharide, Group B streptococcus polysaccharide, and *N. meningitidis* polysaccharide.

18. A method according to claim **14**, wherein the protein is selected from the group consisting of bovine serum albumin, pertussis toxoid, tetanus toxoid, malaria-derived peptide, an antibody, a toxoid, and a lipoprotein.

19. A method according to claim **14**, wherein the immunogenic construct is a conjugate selected from the group consisting of PT-Pn, PT-PRP, TT-Pn, antibody-dextran, and peptide-TT-Pn.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.: 5,693,326

DATED: December 2, 1997

INVENTOR(S): Lees et al.

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

CLAIM 13, Col. 30, L. 61 change "by;" to --by:--.

Face of the patent, change "The portion of the term of this patent subsequent to Mar. 22, 2012, has been disclaimed" to - -The portion of the term of this patent subsequent to the expiration date of U.S. Patent No. 5,651,971 has been disclaimed- -.

Signed and Sealed this

Twenty-fourth Day of October, 2000

Attest:

Q. TODD DICKINSON

Attesting Officer

Director of Patents and Trademarks

Exhibit B

Assignment of the '326 patent from the inventors to
the Uniformed Services University of the Health Sciences
and from the Uniformed Services University of the Health Sciences
to the Foundation

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

04995.0005.02

101375591A

JULY 31, 2000

PTAS

FINNEGAN, HENDERSON, FARABOW ET AL.
JEAN BURKE FORDIS
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WASHINGTON, DC 20005-3315

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RECORDATION DATE: 05/19/2000

REEL/FRAME: 010822/0353
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
LEES, ANDREW

DOC DATE: 02/01/2000

ASSIGNOR:
MOND, JAMES J.

DOC DATE: 02/01/2000

ASSIGNOR:
SNAPPER, CLIFFORD M.

DOC DATE: 02/07/2000

ASSIGNEE:
UNIFORMED SERVICES UNIVERSITY OF
THE HEALTH SCIENCES
WASHINGTON, D.C. 20310

SERIAL NUMBER: 08456694
PATENT NUMBER: 5693326

FILING DATE: 06/01/1995
ISSUE DATE: 12/02/1997

SHAREILL COLES, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

06-07-2000

101375591

FORM PTO-1594
1-31-92

U.S. Department of Commerce
Patent and Trademark Office
Attorney Docket No. 4995.0005-02

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1. Name of conveying party(ies):

Dr. Andrew Lees
Dr. James J. Mond
Dr. Clifford M. Snapper

OIPE JC68 MAY 19 2000 PATENT & TRADEMARK OFFICE

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: Uniformed Services University of the Health Sciences

Internal Address:

Street Address:

City: Washington

State: D.C. Zip Code: 20310

3. Nature of conveyance:

MRD 5/19/00

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other

Execution Dates: February 1, 2000; February 7, 2000

Additional name(s) & Address(es) attached?

☐ Yes ☒ No

4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application:

A. Patent Application Number(s):

B. Patent Number(s):

5,693,326

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Jean Burke Fordis

Internal Address: FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

06/06/2000 JSHABAZZ 00000094 5693326

01 FC:581 40.00 OP

Street Address: 1300 I Street, N.W.

City: Washington

State: DC Zip: 20005-3315

6. Total number of applications and registrations involved: 1

7. Total fee (37 CFR 3.41): $40

☒ Enclosed (Please charge deficiency to deposit account)

☒ Authorized to be charged to deposit account

8. Deposit account number: 06-0916

9. Statement and signature.
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Brian M. Burn, Reg. No. 44,455

Signature

May 19, 2000

Date

Total number of pages including cover sheet, attachments and documents: 3

SOLE/JOINT INVENTION
(Worldwide Rights)

ASSIGNMENT

WHEREAS We, the below named inventors, hereinafter referred to as Assignors, have made an invention entitled:

PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS

for which was filed a continuation-in-part application on September 22, 1993 (Serial No. 08/124,491), a continuation on March 22, 1995 (Serial No. 08/408,717), a continuation on June 1, 1995 (Serial No. 08/456,694), and which issued as U.S. Patent No. 5,693,326 on December 2, 1997; and

WHEREAS, the United States of America, as represented by the Secretary of the Army, whose post office address is Washington, D.C. 20310 (hereinafter referred to as Assignee), is desirous of securing the entire right, title, and interest in and to this invention in all countries throughout the world, and in and to the United States Letters Patent;

AND WHEREAS, Dr. Andrew Lees has already assigned his rights directly to the Jackson Foundation by virtue of an Assignment executed on August 18, 1995, but said Assignment was made in error, and was void ab initio, because Dr. Lees is obligated to assign his rights to the United States of America, as represented by the Secretary of the Army, which by virtue of a separate agreement with the Jackson Foundation would thereafter assign its rights to the Jackson Foundation;

NOW THEREFORE, be it known that for and in consideration of the sum of One Dollar ($1.00) in hand paid and other good and valuable consideration the receipt of which from Assignee is hereby acknowledged, the Assignors have sold, assigned, transferred, and set over, and do hereby sell, assign, transfer, and set over unto the Assignee, its lawful successors and assigns, Assignors' entire right, title, and interest in and to United States Letters Patent No. 5,693,326, issued December 2, 1997, and all reissues thereof, and all rights to claim priority on the basis of the above applications, as well as all rights to claim priority on the basis of this patent, and all applications for Letters Patent which may hereafter be filed for this invention in any foreign country and all Letters Patent which may be granted on this invention in any foreign country, and all extensions, renewals, and reissues thereof; and Assignors hereby authorize and request the Commissioner of Patents and Trademarks of the United States and any official of any foreign country whose duty it is to issue patents on applications as described above, to issue all Letters Patent for this invention to Assignee, its successors and assigns, in accordance with the terms of this Assignment;

AND, ASSIGNORS HEREBY covenant that Assignors have the full right to convey the interest assigned by this Assignment, and Assignors have not executed and will not execute any agreement in conflict with this Assignment;

AND, ASSIGNORS HEREBY further covenant and agree that Assignors will, without further consideration, communicate with Assignee, its successors and assigns, any facts known to Assignors respecting this invention, and testify in any legal proceeding, sign all lawful papers when called upon to do so, execute and deliver any and all papers that may be necessary or desirable to perfect the title to this invention in said Assignee, its successors or assigns, execute all divisional, continuation, and reissue applications, make all rightful oaths and generally do everything possible to aid Assignee, its successors and assigns, to obtain and enforce proper patent protection for this invention in the United States and any foreign country, it being understood that any expense incident to the execution of such papers shall be borne by the Assignee, its successors and assigns.

IN TESTIMONY WHEREOF, Assignors have hereunto set my hands.

County of Montgomery)
State of Maryland)
ss.

Dr. Andrew Lees
1910 Glen Ross Road
Silver Spring, Maryland 20910

(Signature)

Dated: 2/1/00

Subscribed and sworn to before me this 15 day of February, 2000

, Notary Public

ALPHONSUS HENRY
NOTARY PUBLIC STATE OF MARYLAND
My Commission Expires 9/24/00

SOLE/JOINT INVENTION
(Worldwide Rights)

County of Montgomery)
State of Maryland)

ss.

Dr. James J. Mond
427 Northwest Drive
Silver Spring, Maryland 20910

(Signature)

Dated: 1.2.00

ALPHONSUS HENRY
NOTARY PUBLIC STATE OF MARYLAND
My Commission Expires [illegible]

Subscribed and sworn to before me this 1st day of February, 2000

[signature], Notary Public

County of [illegible])
State of Maryland)

ss.

Dr. Clifford M. Snapper
7904 Ivymount Terrace
Potomac, Maryland 20854

(Signature)

Dated: 2/7/00

Subscribed and sworn to before me this 7th day of February 2000

[signature] Notary Public

My Commission expires 4-1-00

ANNA F CONNELLEE
NOTARY PUBLIC
CARROLL CO., MARYLAND

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RECORDATION DATE: 05/19/2000

REEL/FRAME: 010822/0375
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES

DOC DATE: 05/09/2000

ASSIGNEE:
HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, THE
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SERIAL NUMBER: 08456694
PATENT NUMBER: 5693326

FILING DATE: 06/01/1995
ISSUE DATE: 12/02/1997

TONYA LEE, EXAMINER
ASSIGNMENT DIVISION
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MLP 5-19-00

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: The Henry M. Jackson Foundation for the Advancement of Military Medicine

Internal Address:

Street Address: 1401 Rockville Pike, Suite 600

City: Rockville

State: MD Zip Code: 20852

3. Nature of conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

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Execution Date: May 9, 2000

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4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application:

A. Patent Application Number(s):

MLP

B. Patent Number(s):

5,693,326

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Jean Burke Fordis

Internal Address: FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

06/06/2000 JSHABAZZ 00000095 5693326

01 FC:581 40.00 OP

Street Address: 1300 I Street, N.W.

City: Washington

State: DC Zip: 20005-3315

6. Total number of applications and registrations involved: 1

7. Total fee (37 CFR 3.41): $40

☒ Enclosed (Please charge deficiency to deposit account)

☒ Authorized to be charged to deposit account

8. Deposit account number: 06-0916

9. Statement and signature.
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Brian M. Burn, Reg. No. 44,455

Signature

May 19, 2000

Date

Total number of pages including cover sheet, attachments and documents: 3

ASSIGNMENT

WHEREAS, the UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES, a United States Government Agency (hereinafter referred to as Assignor), by virtue of an assignment from inventors executed on February 1, 2000 and February 7, 2000, has right, title, and interest in an invention entitled:

Producing Immunogenic Constructs Using Soluble Carbohydrates Activated Via Organic Cyanylating Reagents

for which was filed a continuation-in-part application on September 22, 1993 (Serial No. 08/124,491), a continuation on March 22, 1995 (serial No. 08/408,717), a continuation on June 1, 1995 (Serial No. 08/456,694), and which issued a U.S. Patent No. 5,693,326 on December 2, 1997; and

WHEREAS, the HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, a corporation of Rockville, Maryland, whose post office address is 1401 Rockville Pike, Suite 600, Rockville, Maryland 20852 (hereinafter referred to as Assignee), is desirous of securing the right, title, and interest in and to this invention in all countries throughout the world, and in and to the application for United States Letters Patent on this invention and the Letters Patent to be issued upon this application; and

WHEREAS, Congress envisioned that the Assignor should be, among other things, at the forefront of medical teaching, research and care, and, in 1983, in order to facilitate the carrying out of these purposes, established the Assignee; and

WHEREAS, accordingly, the Assignor and the Assignee have entered into an agreement on Patents and Technology whereby the Assignee is the Assignor's primary patent management organization, and this Assignment is in furtherance of this agreement.

NOW THEREFORE, be it known that for and in consideration the receipt of which from Assignee is hereby acknowledged, the Assignor does hereby sell, assign, transfer, and set over unto the Assignee, its lawful successors and assigns, its entire right, title, and interest in and to this invention, and this application, and all divisions, and continuations thereof, and all Letters Patent of the United States which may be granted thereon, and all reissues thereof; and all rights to claim priority on the basis of such application, and all applications for Letters Patent which may hereafter be filed for this invention in any foreign country and all Letters Patent which may be granted on this invention in any foreign country, and hereby authorizes and requests the Commissioner of Patents and Trademarks of the United States and any official of any foreign country whose duty it is to issue patents on applications as described above, to issue all Letters Patent for this invention to Assignee, its successors and assigns, in accordance with the terms of this Assignment.

THE ASSIGNOR DOES HEREBY covenant that it has the full right to convey the interest assigned by this Assignment, and has not executed and will not execute any agreement in conflict with this Assignment; and

THE ASSIGNOR DOES HEREBY further covenant and agree that it will, without further consideration, communicate with Assignee, its successors and assigns, any facts known to it respecting this invention, and testify in any legal proceeding, sign all lawful papers when called upon to do so, execute and deliver any and all papers that may be necessary or desirable to perfect the title to this invention in said Assignee, its successors or assigns, execute all divisional, continuation, and reissue applications, make all rightful oaths and generally do everything possible to aid Assignee, its successors and assigns, to obtain and enforce proper patent protection for this invention, it being understood that any expense incident to the execution of such papers shall be borne by the Assignee, its successors and assigns.

IN TESTIMONY WHEREOF, I have hereunto set my hand.

STATE OF MARYLAND, COUNTY OF MONTGOMERY, SS.

DR. JAMES A. ZIMBLE, PRESIDENT
UNIFORMED SERVICES UNIVERSITY
OF THE HEALTH SCIENCES

Subscribed and sworn to before me this
9th day of May, 2002.

Notary Public

My Commission expires 4-1-04

ANNA F. CONNELLEE NOTARY PUBLIC CARROLL CO., MARYLAND

Exhibit C

Power of Attorney Authorizing Anthony C. Tridico to Act on Behalf of the Foundation

PATENT
Attorney Docket No. 04995.0005-05000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of:)
)
Andrew Lees)
)
Patent No.: 5,693,326)
)
Issued: December 2, 1997)
)
For: PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

REVOCATION OF POWER OF ATTORNEY
STATEMENT UNDER 37 C.F.R. § 3.73(b)
AND GRANT OF NEW POWER OF ATTORNEY

The undersigned, a representative authorized to sign on behalf of the assignee owning all of the interest in this patent, hereby revokes all previous powers of attorney or authorization of agent granted in this patent before the date of execution hereof.

As required by 37 C.F.R. § 3.73(b), the undersigned verifies that The Henry M. Jackson Foundation for the Advancement of Military Medicine is the assignee of its entire right, title, and interest in the patent identified above by virtue of an assignment from the inventors to the Uniformed Services University of the Health Sciences, recorded at Reel 010822, Frame 0353, on May 19, 2000, and an assignment from the Uniformed Services University of the Health Sciences to The Henry M. Jackson

Foundation for the Advancement of Military Medicine, recorded at Reel 010822, Frame 0375, on May 19, 2000.

The undersigned representative of the Assignee hereby grants its power of attorney to the patent practitioners associated with **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., Customer Number 22,852,** to transact all business in the Patent and Trademark Office connected with this patent.

Please send all future correspondence concerning this patent to Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., Customer No. 22,852.

Dated: August 7, 2012

By: _____
John W. Lowe
President
The Henry M. Jackson Foundation for the Advancement of Military Medicine

Exhibit D

Approved package insert for MENHIBRIX™

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use MENHIBRIX safely and effectively. See full prescribing information for MENHIBRIX.

MENHIBRIX (Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine)
Solution for Intramuscular Injection
Initial U.S. Approval: 2012

----------INDICATIONS AND USAGE----------

MENHIBRIX is a vaccine indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroups C and Y and *Haemophilus influenzae* type b. MENHIBRIX is approved for use in children 6 weeks of age through 18 months of age. (1)

---------- DOSAGE AND ADMINISTRATION ----------

Four doses (0.5 mL each) by intramuscular injection at 2, 4, 6, and 12 through 15 months of age. The first dose may be given as early as 6 weeks of age. The fourth dose may be given as late as 18 months of age. (2.3)

---------- DOSAGE FORMS AND STRENGTHS ----------

Solution for injection supplied as a single-dose vial of lyophilized vaccine to be reconstituted with the accompanying vial of saline diluent. A single dose after reconstitution is 0.5 mL. (3)

----------CONTRAINDICATIONS----------

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any meningococcal-, *H. influenzae* type b-, or tetanus toxoid-containing vaccine or any component of MENHIBRIX. (4)

---------- WARNINGS AND PRECAUTIONS----------

- If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including MENHIBRIX, should be based on consideration of the potential benefits and possible risks. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including MENHIBRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including MENHIBRIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination. (5.3)

---------- ADVERSE REACTIONS ----------

Rates of local injection site pain, redness, and swelling ranged from 15% to 46% depending on reaction and specific dose in schedule. Commonly reported systemic events included irritability (62% to 71%), drowsiness (49% to 63%), loss of appetite (30% to 34%), and fever (11% to 26%) (specific rate depended on the event and dose in the schedule). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------DRUG INTERACTIONS----------

Do not mix MENHIBRIX with any other vaccine in the same syringe or vial. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 06/2012

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

MENHIBRIX® is indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroups C and Y and *Haemophilus influenzae* type b. MENHIBRIX is approved for use in children 6 weeks of age through 18 months of age.

2 DOSAGE AND ADMINISTRATION

2.1 Reconstitution

MENHIBRIX is to be reconstituted only with the accompanying saline diluent. The reconstituted vaccine should be a clear and colorless solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

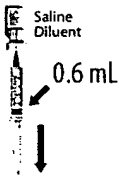


Figure 1. Cleanse both vial stoppers. Withdraw 0.6 mL of saline from diluent vial.

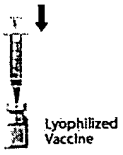


Figure 2. Transfer saline diluent into the lyophilized vaccine vial.

Figure 3. Shake the vial well.

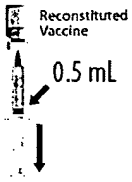


Figure 4. After reconstitution, withdraw 0.5 mL of reconstituted vaccine and administer **intramuscularly**.

2.2 Administration

For intramuscular use only. Do not administer this product intravenously, intradermally, or subcutaneously.

After reconstitution, administer MENHIBRIX immediately.

Use a separate sterile needle and sterile syringe for each individual. The preferred administration site is the anterolateral aspect of the thigh for most infants younger than 1 year of age. In older children, the deltoid muscle is usually large enough for an intramuscular injection.

2.3 Dose and Schedule

A 4-dose series, with each 0.5-mL dose given by intramuscular injection at 2, 4, 6, and 12 through 15 months of age. The first dose may be given as early as 6 weeks of age. The fourth dose may be given as late as 18 months of age.

3 DOSAGE FORMS AND STRENGTHS

MENHIBRIX is a solution for injection supplied as a single-dose vial of lyophilized vaccine to be reconstituted with the accompanying vial of saline diluent. A single dose after reconstitution is 0.5 mL.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any meningococcal-, *H. influenzae* type b-, or tetanus toxoid-containing vaccine or any component of this vaccine is a contraindication to administration of MENHIBRIX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including MENHIBRIX, should be based on consideration of the potential benefits and possible risks.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including MENHIBRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including MENHIBRIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination.

5.4 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the patient's immunization history for possible vaccine hypersensitivity. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

5.5 Altered Immunocompetence

Safety and effectiveness of MENHIBRIX in immunosuppressed children have not been evaluated. If MENHIBRIX is administered to immunosuppressed children, including children receiving immunosuppressive therapy, the expected immune response may not be obtained.

5.6 Tetanus Immunization

Immunization with MENHIBRIX does not substitute for routine tetanus immunization.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the

clinical trials of another vaccine, and may not reflect the rates observed in practice. There is the possibility that broad use of MENHIBRIX could reveal adverse reactions not observed in clinical trials.

A total of 7,521 infants received at least one dose of MENHIBRIX in 6 clinical studies.[1-6] In 5 of these studies, 6,686 children received 4 consecutive doses of MENHIBRIX.[2-6] Across all studies, approximately half of participants were female; 50% were white, 41% were Hispanic, 4% were black, 1% were Asian and 4% were of other racial/ethnic groups.

Two randomized, controlled, pivotal trials enrolled participants to receive 4 doses of MENHIBRIX or a monovalent Haemophilus b Conjugate (Hib) vaccine, administered at 2, 4, 6, and 12 to 15 months of age (Study 009/010[5] and Study 011/012[6]). Together, these trials evaluated safety in 8,571 infants who received at least one dose of MENHIBRIX (N = 6,414) or Hib vaccine (N = 2,157).[5,6]

In Study 009/010[5], conducted in the United States, Australia, and Mexico, 4,180 infants were randomized 3:1 to receive MENHIBRIX or a control US-licensed Hib vaccine. Safety data are available for 3,136 infants who received MENHIBRIX and 1,044 infants who received a control Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate) (PRP-T, manufactured by Sanofi Pasteur SA) at 2, 4, and 6 months of age. For dose 4 administered at 12 to 15 months of age, safety data are available for 2,769 toddlers who received MENHIBRIX and 923 toddlers who received a control Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate) (PRP-OMP, manufactured by Merck and Co., Inc.). With doses 1, 2, and 3 of MENHIBRIX or PRP-T, infants concomitantly received PEDIARIX® [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine] and Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein) (PCV7, manufactured by Wyeth Pharmaceuticals, Inc.). With dose 4 of MENHIBRIX or PRP-OMP, toddlers concomitantly received PCV7, Measles, Mumps, and Rubella Virus Vaccine Live (MMR, manufactured by Merck & Co., Inc.), and Varicella Virus Vaccine Live (manufactured by Merck & Co., Inc.).

Data on solicited adverse events were collected by parents/guardians using standardized forms for 4 consecutive days following vaccination with MENHIBRIX or control Hib vaccine (i.e., day of vaccination and the next 3 days).[5] Children were monitored for unsolicited adverse events that occurred in the 31-day period following vaccination and were monitored for serious adverse events; new onset chronic disease, rash, and conditions prompting emergency department visits or physician office visits during the entire study period (6 months following the last vaccine administered). Among participants in both groups, 66% were from the United States, 19% were from Mexico, and 14% were from Australia. Forty-eight percent of participants were female; 64% were white, 22% were Hispanic, 6% were black, 1% were Asian, and 7% were of other racial/ethnic groups.

In the second pivotal study (Study 011/012[6]), conducted in the United States and Mexico and evaluating the same vaccines and vaccination schedule, participants were monitored for serious adverse events, new onset chronic disease, rash, and conditions prompting emergency

department visits during the entire study period (6 months following the last vaccine administered). Among participants in both groups, 30% were from the United States and 70% were from Mexico.

In addition to the pivotal studies, safety data are available from 4 studies which either did not include a fourth dose of MENHIBRIX[1], used a dosing regimen not approved in the United States[2,3], or incorporated a comparator vaccine which was not licensed in the United States.[4] In these studies, participants were monitored for unsolicited adverse events and serious adverse events occurring in the 31–day period following vaccination. In 2 of these studies[3,4], participants were monitored for serious adverse events, new onset chronic disease, rash, and conditions prompting emergency department visits or physician office visits through 6 months after the last vaccination.

Solicited Adverse Events: The reported frequencies of solicited local and systemic adverse events from US participants in Study 009/010 are presented in Table 1.[5] Because of differences in reported rates of solicited adverse events between US and non-US participants, only the solicited adverse event data in US participants are presented. Among the US participants included in Table 1, 48% were female; 76% were white, 10% were black, 4% were Hispanic, 2% were Asian, and 8% were of other racial/ethnic groups.

Table 1. Percentage of US Children from Study 009/010 With Solicited Local and General Adverse Events within 4 Days of Vaccination[a] With MENHIBRIX or Haemophilus b Conjugate Vaccine (Total Vaccinated Cohort)

	MENHIBRIX[b]				Haemophilus b Conjugate Vaccine[b,c]			
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 1	Dose 2	Dose 3	Dose 4
Local[d]								
N	2,009	1,874	1,725	1,533	659	612	569	492
Pain, any	46.2	44.6	41.4	42.1	61.6	52.8	49.9	50.4
Pain, grade 3[e]	3.7	3.3	2.3	1.6	11.4	5.1	3.0	5.3
Redness, any	20.6	31.0	35.5	34.6	27.9	33.7	42.2	46.7
Redness, >30 mm	0.1	0.3	0.1	0.7	1.8	0.3	0.4	1.2
Swelling, any	14.7	20.4	23.8	25.4	20.5	20.8	28.6	31.7
Swelling, >30 mm	0.5	0.3	0.3	0.6	1.5	0.2	0.4	0.8
Systemic								
N	2,008-2,009	1,871	1,723	1,535-1,536	659	609-610	569	493-494
Irritability	67.5	70.8	65.8	62.1	76.9	75.1	65.4	66.1
Irritability, grade 3[f]	3.7	4.8	3.3	2.5	7.4	5.6	4.2	4.3
Drowsiness, any	62.8	57.7	49.5	48.7	66.9	61.8	52.4	48.5
Drowsiness, grade 3[g]	2.7	3.2	1.7	2.1	2.7	2.6	1.4	2.0
Loss of appetite, any	33.8	32.1	30.1	32.1	37.6	33.6	30.2	32.5
Loss of appetite, grade 3[h]	0.5	0.7	0.5	1.1	0.3	0.7	1.1	2.2
Fever, ≥100.4°F[i]	18.9	25.9	23.0	11.0	21.4	28.2	23.7	12.6
Fever, ≥102.2°F[i]	1.1	1.9	3.2	1.5	0.9	2.6	2.8	2.0
Fever, >104°F[i]	0.0	0.1	0.3	0.3	0.0	0.0	0.4	0.2

Total Vaccinated Cohort = all participants who received at least one dose of either vaccine.

N = number of participants who completed the symptom sheet for a given symptom at the specified dose.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] Co-administered with PEDIARIX and PCV7 at doses 1, 2, 3 and PCV7, MMR and varicella vaccines at dose 4.

[c] US-licensed monovalent Haemophilus b Conjugate Vaccine manufactured by Sanofi Pasteur SA for doses 1, 2, and 3 (PRP-T) and by Merck & Co., Inc for dose 4 (PRP-OMP).

[d] Local reactions at the injection site for MENHIBRIX or Haemophilus b Conjugate Vaccine.

[e] Cried when limb was moved/spontaneously painful.

[f] Crying that could not be comforted/prevented normal daily activities.

[g] Prevented normal daily activities.

[h] Not eating at all.

[i] Across both treatment groups, 54%, 56%, and 59% of participants had temperatures measured rectally following doses 1, 2, and 3, respectively; 45%, 44%, and 40% of participants had temperatures measured by the axillary route for doses 1, 2, and 3, respectively. For dose 4, >90% of participants had temperatures measured via the axillary route.

The reported rates of some solicited adverse events in participants from Australia and Mexico varied from those in the United States.[5] For example, in Australia, pain after dose 1 was reported in 28.4% of participants who received MENHIBRIX and 33.3% of control participants, while in Mexico pain after dose 1 was reported in 73.7% of participants who received MENHIBRIX and 79.4% of control participants. Fever after dose 1 was reported in 10.4% of participants who received MENHIBRIX and 10.7% of control participants in Australia, while it was reported in 44.0% of participants who received MENHIBRIX and 35.7% of control participants in Mexico. The reported incidences of pain and fever in US participants after dose 1 are provided in Table 1.

Unsolicited Adverse Events: Among participants who received MENHIBRIX or Hib control vaccine co-administered with US-licensed vaccines at 2, 4, 6 and 12 to 15 months of age[1,3-5], the incidence of unsolicited adverse events reported within the 31-day period following study vaccination (doses 1, 2, and 3) was comparable between MENHIBRIX (61.9%; 2,578/4,166) and PRP-T (62.5%; 1,042/1,666). The incidence of unsolicited adverse events reported within the 31-day period following dose 4 was also comparable between MENHIBRIX (42.5%; 1,541/3,630) and PRP-OMP (41.4%; 520/1,257).

Serious Adverse Events: Following doses 1, 2, and 3[1,3-6], 1.8% (137/7,444) of participants who received MENHIBRIX and 2.1% (59/2,779) of participants who received PRP-T reported at least one serious adverse event within the 31-day period. Up to 6 months following the last vaccine administered (doses 1, 2, and 3) or until administration of dose 4[3-6], 4.8% (365/7,362) of participants who received MENHIBRIX and 5.0% (134/2,697) of participants in the PRP-T group reported at least one serious adverse event.

Following dose 4[3-6], 0.5% (35/6,640) of participants who received MENHIBRIX and 0.5% (12/2,267) of participants who received PRP-OMP reported at least one serious adverse event within the 31-day period. Up to 6 months following the last vaccine administered (dose 4), 2.5% (165/6,640) of participants who received MENHIBRIX and 2.0% (46/2,267) of participants who received PRP-OMP reported at least one serious adverse event.

6.2 Postmarketing Experience

The following adverse events have been spontaneously reported during post-approval use of HIBERIX® (Haemophilus b Conjugate Vaccine [Tetanus Toxoid Conjugate]) in the United States and other countries. These events are relevant because the Haemophilus b capsular polysaccharide tetanus toxoid conjugate is included as a component antigen in both MENHIBRIX and HIBERIX. Because these events are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or to establish a causal relationship to vaccine exposure.

The following adverse events were included based on one or more of the following factors: seriousness, frequency of reporting, or strength of evidence for a causal relationship to HIBERIX.

General Disorders and Administration Site Conditions: Extensive swelling of the vaccinated limb, injection site induration.

Immune System Disorders: Allergic reactions (including anaphylactic and anaphylactoid reactions), angioedema.

Nervous System Disorders: Convulsions (with or without fever), hypotonic-hyporesponsive episode, somnolence, syncope or vasovagal responses to injection.

Respiratory, Thoracic, and Mediastinal Disorders: Apnea.

Skin and Subcutaneous Tissue Disorders: Rash, urticaria.

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

In clinical studies, MENHIBRIX was administered concomitantly with routinely recommended pediatric US-licensed vaccines *[see Adverse Reactions (6.1) and Clinical Studies (14.2)]*.

If MENHIBRIX is administered concomitantly with other injectable vaccines, they should be given with separate syringes and at different injection sites. MENHIBRIX should not be mixed with any other vaccine in the same syringe or vial.

7.2 Interference With Laboratory Tests

Haemophilus b capsular polysaccharide derived from Haemophilus b Conjugate Vaccines has been detected in the urine of some vaccinees.[7] Urine antigen detection may not have a diagnostic value in suspected disease due to *H. influenzae* type b within 1 to 2 weeks after receipt of a *H. influenzae* type b-containing vaccine, including MENHIBRIX.

7.3 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to MENHIBRIX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Animal reproduction studies have not been conducted with MENHIBRIX. It is also not known whether MENHIBRIX can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity.

8.4 Pediatric Use

Safety and effectiveness of MENHIBRIX in children younger than 6 weeks of age and in children 19 months to 16 years of age have not been established.

11 DESCRIPTION

MENHIBRIX (Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine), for intramuscular injection, is supplied as a sterile, lyophilized powder which is reconstituted at the time of use with the accompanying saline diluent. MENHIBRIX contains *Neisseria meningitidis* serogroup C and Y capsular polysaccharide antigens and Haemophilus b capsular polysaccharide (polyribosyl-ribitol-phosphate [PRP]). The *Neisseria meningitidis* C strain and Y strain are grown in semi-synthetic media and undergo heat inactivation and purification. The PRP is a high molecular weight polymer prepared from the *Haemophilus influenzae* type b strain 20,752 grown in a synthetic medium that undergoes heat inactivation and purification. The tetanus toxin, prepared from *Clostridium tetani* grown in a semi-synthetic medium, is detoxified with formaldehyde and purified. Each capsular polysaccharide is individually covalently bound to the inactivated tetanus toxoid. After purification, the conjugate is lyophilized in the presence of sucrose as a stabilizer. The diluent for MENHIBRIX is a sterile saline solution (0.9% sodium chloride) supplied in vials.

When MENHIBRIX is reconstituted with the accompanying saline diluent, each 0.5-mL dose is formulated to contain 5 mcg of purified *Neisseria meningitidis* C capsular polysaccharide conjugated to approximately 5 mcg of tetanus toxoid, 5 mcg of purified *Neisseria meningitidis* Y capsular polysaccharide conjugated to approximately 6.5 mcg of tetanus toxoid, and 2.5 mcg of purified Haemophilus b capsular polysaccharide conjugated to approximately 6.25 mcg of tetanus toxoid. Each dose also contains 96.8 mcg of Tris (trometamol)-HCl, 12.6 mg of sucrose, and ≤0.72 mcg of residual formaldehyde. MENHIBRIX does not contain preservatives. The vial stoppers do not contain latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Neisseria meningitidis: The presence of bactericidal anti-capsular meningococcal antibodies has been associated with protection from invasive meningococcal disease.[8] MENHIBRIX induces production of bactericidal antibodies specific to the capsular polysaccharides of serogroups C and Y.

Haemophilus influenzae type b: Specific levels of antibodies to PRP (anti-PRP) have been shown to correlate with protection against invasive disease due to *H. influenzae* type b. Based on data from passive antibody studies[9] and a clinical efficacy study with unconjugated *Haemophilus* b polysaccharide vaccine[10], an anti-PRP concentration of 0.15 mcg/mL has been accepted as a minimal protective level. Data from an efficacy study with unconjugated *Haemophilus* b polysaccharide vaccine indicate that an anti-PRP concentration of ≥1.0 mcg/mL predicts protection through at least a 1-year period.[11,12] These antibody levels have been used to evaluate the effectiveness of *H. influenzae* type b-containing vaccines, including MENHIBRIX.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

MENHIBRIX has not been evaluated for carcinogenic or mutagenic potential, or for impairment of fertility.

14 CLINICAL STUDIES

14.1 Immunological Evaluation

In Study 009/010[5] the immune response to MENHIBRIX and control vaccines was evaluated in a subset of US participants. In this clinical study, MENHIBRIX and Hib control vaccines were administered concomitantly with routinely recommended US-licensed vaccines *[see Adverse Reactions (6.1)]*. Among participants in the ATP immunogenicity cohort for both vaccine groups combined, 47% were female; 81% of participants were white, 8% were black, 4% were Hispanic, 1% were Asian, and 6% were of other racial/ethnic groups.

Study objectives included evaluation of *N. meningitidis* serogroups C (MenC) and Y (MenY) as measured by serum bactericidal assay using human complement (hSBA) and antibodies to PRP as measured by enzyme-linked immunosorbent assay (ELISA) in sera obtained approximately one month (range 21 to 48 days) after dose 3 of MENHIBRIX or PRP-T and approximately 6 weeks (range 35 to 56 days) after dose 4 of MENHIBRIX or PRP-OMP. The hSBA-MenC and hSBA-MenY geometric mean antibody titers (GMTs) and the percentage of participants with hSBA-MenC and hSBA-MenY levels ≥1:8 are presented in Table 2. Anti-PRP geometric mean antibody concentrations (GMCs) and the percentage of participants with anti-PRP levels ≥0.15 mcg/mL and ≥1.0 mcg/mL are presented in Table 3.

Table 2. Bactericidal Antibody Responses Following MENHIBRIX (One Month After Dose 3 and 6 Weeks After Dose 4) in US Children Vaccinated at 2, 4, 6, and 12 to 15 Months of Age (ATP Cohort for Immunogenicity)

	MENHIBRIX Post-Dose 3	MENHIBRIX Post-Dose 4
hSBA-MenC	N = 491	N = 331
% ≥1:8	98.8	98.5[a]
95% CI	97.4, 99.6	96.5, 99.5
GMT	968	2040
95% CI	864, 1084	1746, 2383
hSBA-MenY	N = 481	N = 342
% ≥1:8	95.8	98.8[a]
95% CI	93.7, 97.4	97.0, 99.7
GMT	237	1390
95% CI	206, 272	1205, 1602

ATP = according to protocol; CI = confidence interval; GMT = geometric mean antibody titer.

N = number of US children eligible for inclusion in the ATP immunogenicity cohort for whom serological results were available for the post-dose 3 and post-dose 4 immunological evaluations.

[a] Acceptance criteria were met (lower limit of 95% CI for the percentage of participants with hSBA-MenC and hSBA-MenY titers ≥1:8 ≥90% following 4 doses).

Table 3. Comparison of anti-PRP Responses Following MENHIBRIX or Haemophilus b Conjugate Vaccine[a] (One Month After Dose 3 and 6 Weeks After Dose 4) in US Children Vaccinated at 2, 4, 6, and 12 to 15 Months of Age (ATP Cohort for Immunogenicity)

	Post-Dose 3		Post-Dose 4	
	MENHIBRIX	PRP-T	MENHIBRIX	PRP-OMP
Anti-PRP	N = 518	N = 171	N = 361	N = 126
% ≥0.15 mcg/mL	100	98.2	100	100
95% CI	99.3, 100	95.0, 99.6	99.0, 100	97.1, 100
% ≥1.0 mcg/mL	96.3[b]	91.2	99.2[b]	99.2
95% CI	94.3, 97.8	85.9, 95.0	97.6, 99.8	95.7, 100
GMC (mcg/mL)	11.0	6.5	34.9	20.2
95% CI	10.0, 12.1	5.3, 7.9	30.7, 39.6	16.4, 24.9

ATP = according to protocol; anti-PRP = antibody concentrations to *H. influenzae* capsular polysaccharide; CI = confidence interval; GMC = geometric mean antibody concentration.

N = number of US children eligible for inclusion in the ATP immunogenicity cohort for whom serological results were available for the post-dose 3 and post-dose 4 immunological evaluations.

[a] US-licensed monovalent Haemophilus b Conjugate Vaccine for doses 1, 2, and 3 (PRP-T) and for dose 4 (PRP-OMP).

[b] Non-inferiority was demonstrated (lower limit of 95% CI on the group difference of MENHIBRIX minus Haemophilus b Conjugate Vaccine ≥-10%).

14.2 Concomitant Vaccine Administration

In participants who received MENHIBRIX concomitantly with PEDIARIX and PCV7 at 2, 4, and 6 months of age, there was no evidence for reduced antibody response to pertussis antigens (GMC to pertussis toxin, filamentous hemagglutinin, and pertactin), diphtheria toxoid (antibody levels ≥0.1 IU/mL), tetanus toxoid (antibody levels ≥0.1 IU/mL), poliovirus types 1, 2, and 3 (neutralizing antibody levels ≥1:8 to each virus), hepatitis B (anti-hepatitis B surface antigen ≥10 mIU/mL) or PCV7 (antibody levels ≥0.2 mcg/mL and GMC to each serotype) relative to the response in control participants administered PRP-T concomitantly with PEDIARIX and PCV7. The immune responses to PEDIARIX[3,5] and PCV7[3] were evaluated one month following dose 3.

There was no evidence for interference in the immune response to MMR and varicella vaccines (initially seronegative participants with anti-measles ≥200 mIU/mL, anti-mumps ≥51 ED_{50}, anti-rubella ≥10 IU/mL, and anti-varicella ≥1:40) administered at 12 to 15 months of age concomitantly with MENHIBRIX and PCV7 relative to these vaccines administered concomitantly with PRP-OMP and PCV7.[4,5] The immune responses to MMR and varicella vaccines were evaluated 6 weeks post-vaccination. Data are insufficient to evaluate potential interference when a fourth PCV7 dose is administered concomitantly with MENHIBRIX at 12 to 15 months of age.

15 REFERENCES

All NCT numbers are as noted in the National Library of Medicine clinical trial database (see www.clinicaltrials.gov).

1. NCT00127855 (001).
2. NCT00129116 (003/004).
3. NCT00129129 (005/006).
4. NCT00134719 (007/008).
5. NCT00289783 (009/010).
6. NCT00345579/NCT00345683 (011/012).
7. Rothstein EP, Madore DV, Girone JAC, et al. Comparison of antigenuria after immunization with three *Haemophilus influenzae* type b conjugate vaccines. *Pediatr Infect Dis J* 1991;10:311-314.
8. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* 1969;129:1307-1326.
9. Robbins JB, Parke JC, Schneerson R, et al. Quantitative measurement of "natural" and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. *Pediatr Res* 1973;7:103-110.
10. Peltola H, Käythy H, Sivonen A, et al. *Haemophilus influenzae* type b capsular polysaccharide vaccine in children: A double-blind field study of 100,000 vaccinees 3 months to 5 years of age in Finland. *Pediatrics* 1977;60:730-737.
11. Käythy H, Peltola H, Karanko V, et al. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1983;147:1100.
12. Anderson P. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1984;149:1034.

16 HOW SUPPLIED/STORAGE AND HANDLING

MENHIBRIX is available in single-dose vials of lyophilized vaccine, accompanied by vials containing 0.85 mL of saline diluent (packaged without syringes or needles).

Supplied as package of 10 doses (NDC 58160-801-11):

NDC 58160-809-01 Vial of lyophilized vaccine in Package of 10: NDC 58160-809-05

NDC 58160-813-01 Vial of saline diluent in Package of 10: NDC 58160-813-05

16.1 Storage Before Reconstitution

Lyophilized vaccine vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light.

Diluent: Store refrigerated or at controlled room temperature between 2° and 25°C (36° and 77°F). Do not freeze. Discard if the diluent has been frozen.

16.2 Storage After Reconstitution

After reconstitution, administer MENHIBRIX immediately. Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

- Inform parents or guardians of the potential benefits and risks of immunization with MENHIBRIX, and of the importance of completing the immunization series.
- Inform parents or guardians about the potential for adverse reactions that have been temporally associated with administration of MENHIBRIX or other vaccines containing similar components.
- Instruct parents or guardians to report any adverse events to their healthcare provider.
- Give parents or guardians the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

HIBERIX, MENHIBRIX, and PEDIARIX are registered trademarks of GlaxoSmithKline.

Manufactured by **GlaxoSmithKline Biologicals**
Rixensart, Belgium, US License 1617, and
Distributed by **GlaxoSmithKline**
Research Triangle Park, NC 27709

MNX:1PI

Exhibit E

FDA Approval Letter

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852-1448

Our STN: BL 125363/0

GlaxoSmithKline Biologicals
Attention: Jody Ann Gould, Ph.D.
2301 Renaissance Boulevard
P.O. Box 61540
King of Prussia, PA 19406-2772

June 14, 2012

Dear Dr. Gould:

We have approved your biologics license application for Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine (MenHibrix) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine under your existing Department of Health and Human Services U.S. License No. 1617. Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine is indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroups C and Y and *Haemophilus influenzae* type b. MenHibrix is approved for use in children 6 weeks of age through 18 months of age.

The review of this product is associated with the following National Clinical Trial (NCT) numbers: NCT00127855, NCT00129116, NCT00129129, NCT00134719, NCT00289783, NCT00345579, NCT00345683, NCT00359983, and NCT00614614.

Under this license, you are approved to manufacture Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine. Commercial manufacturing will be distributed among the following facilities: GlaxoSmithKline Biologicals S.A., located in Wavre, Belgium; GlaxoSmithKline Biologicals S.A., located in Rixensart, Belgium; GlaxoSmithKline Biologicals Kft., located in Gödöllö, Hungary; and Corixa Corporation d/b/a GlaxoSmithKline Biologicals NA, located in Marietta, Pennsylvania, USA. *Neisseria meningitidis* serogroups A and C polysaccharides and *Haemophilus influenzae* b polysaccharide will be manufactured at GlaxoSmithKline Biologicals S.A., located in Wavre, Belgium. Tetanus Toxoid (TT) will be manufactured at GlaxoSmithKline Biologicals Kft., located in Gödöllö, Hungary and GlaxoSmithKline Biologicals S.A., located in Rixensart, Belgium. The manufacture of *Neisseria meningitidis* polysaccharide-TT conjugates and *Haemophilus influenzae* b polysaccharide-TT conjugates will occur at GlaxoSmithKline Biologicals S.A., located in Rixensart, Belgium. Final Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine formulation will occur at GlaxoSmithKline Biologicals S.A., located in Rixensart, Belgium. Final filling and lyophilization for Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine will occur at GlaxoSmithKline Biologicals S.A., located in Wavre, Belgium. Diluent (0.9% sodium chloride) manufacturing, labeling, and

packaging will be conducted by Jubilant HollisterStier LLC, a contract facility, in Spokane, Washington, USA.

Final labeling and packaging for Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine will occur at Corixa Corporation d/b/a GlaxoSmithKline Biologicals NA, located in Marietta, Pennsylvania, USA. Final co-packaging of the lyophilized vaccine and the 0.9% Sodium Chloride Diluent will occur at Corixa Corporation d/b/a GlaxoSmithKline Biologicals NA, located in Marietta, Pennsylvania, USA. You may label your product with the proprietary name MenHibrix. The vaccine will be supplied in packages containing 10 single dose vials of lyophilized Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine and 10 single dose vials of 0.9% Sodium Chloride Diluent for reconstitution.

We did not refer your application to an additional VRBPAC because our review of information submitted in your BLA, including the clinical study design and trial results, did not raise particular concerns or controversial issues which would have benefited from an advisory committee discussion.

The dating period for the lyophilized Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine shall be 36 months from the date of manufacture when stored at 2 to 8°C. The date of manufacture shall be defined as the start date for filling into final containers. Following the final lyophilization, no reprocessing/reworking is allowed without prior approval from the Agency. The dating period for the 0.9% Sodium Chloride Diluent shall be 24 months from the date of manufacture when stored at 2 to 25°C. The date of manufacture for the diluent is defined as the start date for filling into final containers. The dating period for the co-packaged product, lyophilized Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine and the 0.9% Sodium Chloride Diluent, shall be the shorter dating period of the diluent and the lyophilized vaccine.

Please submit final container samples of each kit component in final containers together with protocols showing results of all applicable tests. You may not distribute any lots of product until you receive a notification of release from the Director, Center for Biologic Evaluation and Research.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in, including but not limited to, the manufacturing, testing, packaging or labeling of Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine, or in the manufacturing facilities.

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Director, Office of Compliance and Biologics Quality, Center for Biologics Evaluation and Research, HFM-600, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please provide your final content of labeling in Structured Product Labeling (SPL) format and include the carton and container labels. In addition, please submit three original paper copies for carton and container final printed labeling. All final labeling should be submitted as Product Correspondence to this biologics license application at the time of use (prior to marketing) and include implementation information on FDA Form 356h and FDA Form 2567 as appropriate.

In addition, please submit the final content of labeling (21 CFR 601.14) in SPL format via the FDA automated drug registration and listing system, (eLIST), as described at http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm. Information on submitting SPL files using eLIST may be found in the guidance for industry titled, "SPL Standard for Content of Labeling Technical Qs and As at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf.

You may submit two draft copies of the proposed introductory advertising and promotional labeling with an FDA Form 2253 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Branch, HFM-602, 1401 Rockville Pike, Rockville, MD 20852-1448. You must submit copies of your final advertisement and promotional labeling at the time of initial dissemination or publication, accompanied by Form FDA 2253 (21 CFR 601.12(f)(4)).

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence or substantial clinical experience to support such claims (21 CFR 202.1(e)(6)).

ADVERSE EVENT REPORTING

You must submit adverse experience reports in accordance with the adverse experience reporting requirements for licensed biological products (21 CFR 600.80) and you must submit distribution reports as described in (21 CFR 600.81). You should submit these reports to the Vaccine Adverse Event Reporting System (VAERS), P.O. Box 1100, Rockville, MD 20849-1100, using the pre-addressed form VAERS-1 (http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM164319.pdf). Per 21 CFR 600.2(f), please refer to http://www.fda.gov/AboutFDA/CentersOffices/CBER/ucm106001.htm for updated mailing address information.

PEDIATRIC REQUIREMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are waiving the pediatric study requirement for children 0 to less than 6 weeks of age because the product fails to represent a meaningful therapeutic benefit over initiating vaccination at 6 weeks of age and is unlikely to be used in a substantial number of children 0 to less than 6 weeks of age.

We are waiving the pediatric study requirement for children 19 months to less than 17 years of age because MenHibrix fails to represent a meaningful therapeutic benefit over vaccination with existing vaccines and is unlikely to be used in a substantial number of children in this age group.

We note that you have fulfilled the pediatric study requirement for ages 6 weeks through 18 months of age for this application.

AGREED UPON POSTMARKETING COMMITMENTS

We acknowledge your written commitments as described in your letters of June 4, 2012 and June 12, 2012, as outlined below:

Postmarketing Studies subject to reporting requirements of 21 CFR 601.70.

1. To conduct a Phase IIIb open-label administration (laboratory personnel will be blinded to treatment), parallel-group, controlled, multicenter study to evaluate concomitant administration of MenHibrix with rotavirus, 13-valent pneumococcal conjugate and hepatitis A vaccines administered according to a US recommended vaccine schedule.

   The study is entitled "A phase IIIb, open, randomized, controlled, multicenter study to assess the immunogenicity, safety and reactogenicity of Hib-MenCY-TT (GlaxoSmithKline Biologicals' Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine) when administered concomitantly with *Rotarix* (GlaxoSmithKline Biologicals), *Pediarix* (GlaxoSmithKline Biologicals) and *Prevnar 13* (Pfizer) as compared to *Pentacel* (Sanofi Pasteur) administered concomitantly with *Rotarix* and *Prevnar 13* in healthy infants at 2 and 4 months of age. Subjects will receive a third dose of *Prevnar 13*, *Pediarix* and Hib-MenCY-TT or *Pentacel* at 6 months of age. The study will also assess the immunogenicity, safety and reactogenicity of a fourth dose of Hib-MenCY-TT administered concomitantly with *Havrix* (GlaxoSmithKline Biologicals) compared to *Pentacel* administered concomitantly with *Havrix* at 15 to 18 months of age." The final study protocol will be submitted by December 31, 2012. The study will begin by October 31, 2013. The study will be completed by July 31, 2016. The final study report will be submitted by December 15, 2016.

Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.

2. To implement direct imprinting of the lot number and expiry dating on the combination box to replace the use of an auxiliary label. You will submit the updated outer carton labeling as a prior approval supplement by 30 June 2013.

3. To align the methods to assess the monomeric percentage of TT so that the same method is used by both the Hungarian manufacturing site and the Belgian site on the purified TT bulk. You will submit the Hungarian method and the revised specifications to be used at each site as a prior approval supplement by 31 December 2012.

4. To change the procedures for osmolality testing of MenHibrix final container to reconstitute in saline and to re-evaluate the release specification using this revised procedure. You will submit the updated procedure and revised acceptance criteria as a prior approval supplement by 31 December 2012.

5. To change the procedure for total protein content by Lowry of MenHibrix final container to reconstitute in saline. A comparability study will be performed to assess the impact of the saline reconstitution on the method to determine the need for requalification or revalidation. The impact on the acceptance criteria will also be evaluated. You will submit the revised method and revised acceptance criteria, if applicable, as a prior approval supplement by 31 December 2012.

6. To review the specification limit of MSD by HPLC performed as a QC Release test on Microfluidized MenY polysaccharide once 30 lots have been manufactured and tested. If this review results in a loosening of the specification, you will submit the revised specification as a prior approval supplement by January 2016. If this review results in a tightening of the specification, you will submit the revised specification as a CBE-30 supplement by January 2016. If this review results in no changes to the specification, you will submit the data as a PMC Submission by January 2016.

Please submit clinical protocols to your IND, with a cross-reference letter to this biologics license application, STN BL 125363/0. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA STN BL 125363/0. If the information in the final study report supports a change in the labeling, the final study report should be submitted as a supplement. We may also request a supplement if we think labeling changes are needed. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Commitment Protocol**
- **Postmarketing Study Correspondence**
- **Postmarketing Study Commitment – Final Study Report**
- **Supplement Contains Postmarketing Study Commitments – Final Study Report**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. Label your annual report an "Annual Status Report of Postmarketing Study Commitments." The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e., pending, ongoing, delayed, terminated, or submitted), and
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e., number enrolled to date and the total planned enrollment).

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/PostmarketingPhaseIVCommitments/default.htm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM080569.pdf) for further information.

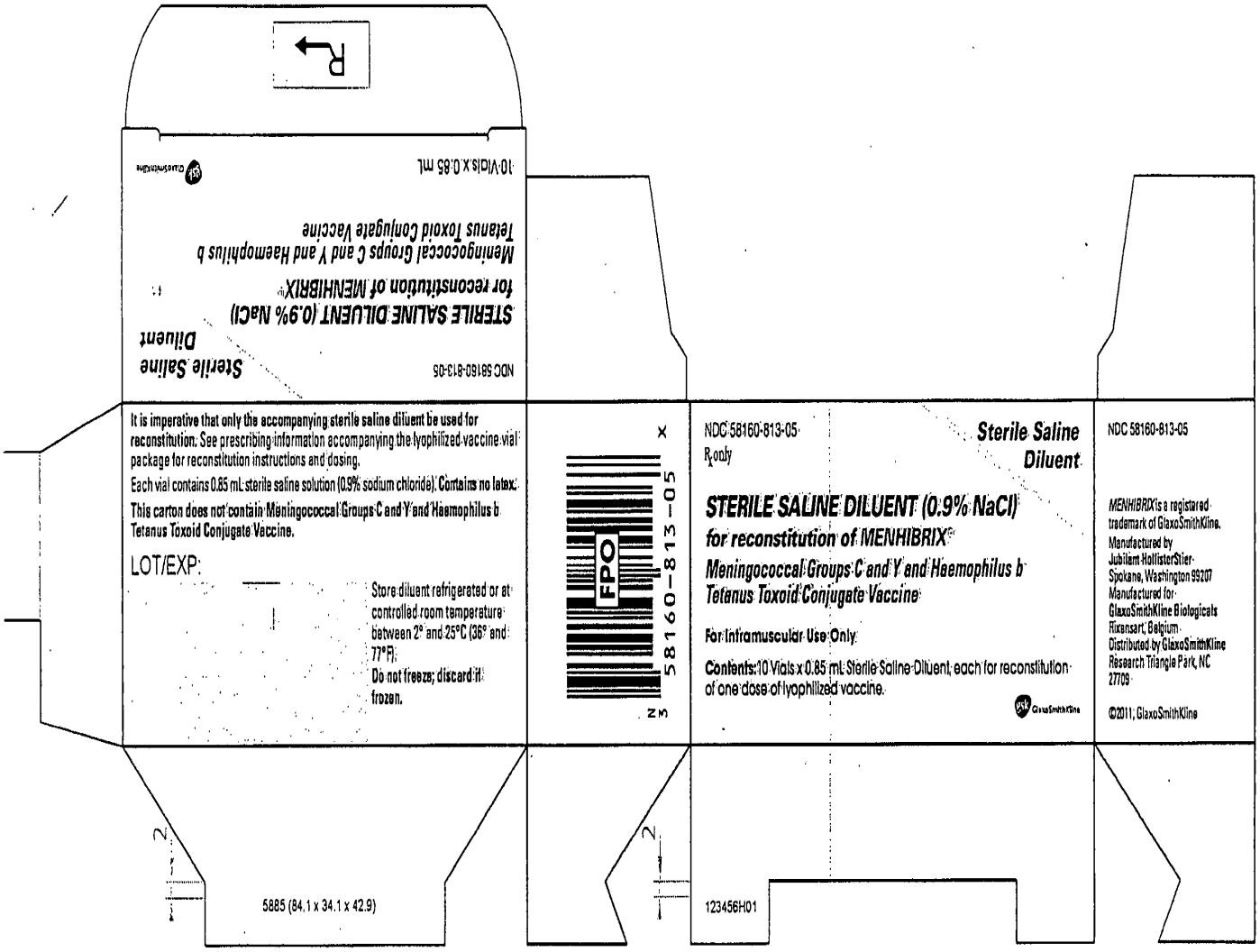
If you have any questions regarding the above, please contact the Regulatory Project Managers, CDR David Staten or Kirk Prutzman, Ph.D., at (301) 796-2640.

Sincerely yours,

Marion F Gruber

Marion Gruber, Ph.D.
Director
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

Attachment: Approved Final Draft Labeling


R only
10 Vials x 0.85 mL
GlaxoSmithKline
Sterile Saline Diluent
NDC 58160-813-05
STERILE SALINE DILUENT (0.9% NaCl)
for reconstitution of MENHIBRIX®
Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine
It is imperative that only the accompanying sterile saline diluent be used for reconstitution. See prescribing information accompanying the lyophilized vaccine vial package for reconstitution instructions and dosing.
Each vial contains 0.85 mL sterile saline solution (0.9% sodium chloride). Contains no latex.
This carton does not contain Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine.
LOT/EXP:
Store diluent refrigerated or at controlled room temperature between 2° and 25°C (36° and 77°F).
Do not freeze; discard if frozen.
FPO
58160-813-05
NDC 58160-813-05
R only
Sterile Saline Diluent
STERILE SALINE DILUENT (0.9% NaCl)
for reconstitution of MENHIBRIX®
Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine
For Intramuscular Use Only
Contents: 10 Vials x 0.85 mL Sterile Saline Diluent, each for reconstitution of one dose of lyophilized vaccine.
GlaxoSmithKline
NDC 58160-813-05
MENHIBRIX is a registered trademark of GlaxoSmithKline.
Manufactured by
Jubilant HollisterStier
Spokane, Washington 99207
Manufactured for
GlaxoSmithKline Biologicals
Rixensart, Belgium
Distributed by GlaxoSmithKline
Research Triangle Park, NC
27709
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2
5885 (84.1 x 34.1 x 42.9)
2
123456H01

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Item Number: 449105	List Colours: (Include sample in fields provided; e.g. spot / 100%CMYK equivalent)	K, 477
Manufacturing Site: GSK-BEL-Wavre-BEWAV-Marietta		
Market or Pack Owner: United States-USA	Technical Reference No(s).: BIO_DRW560 (do NOT include the technical reference doc(s) version no(s).)	RSC A/W Version: 8

Biologicals Additional Information Panel
Material weight: FAMILY 10
Removable parts n.: N/A
Datamatrix code value: 449105BBN

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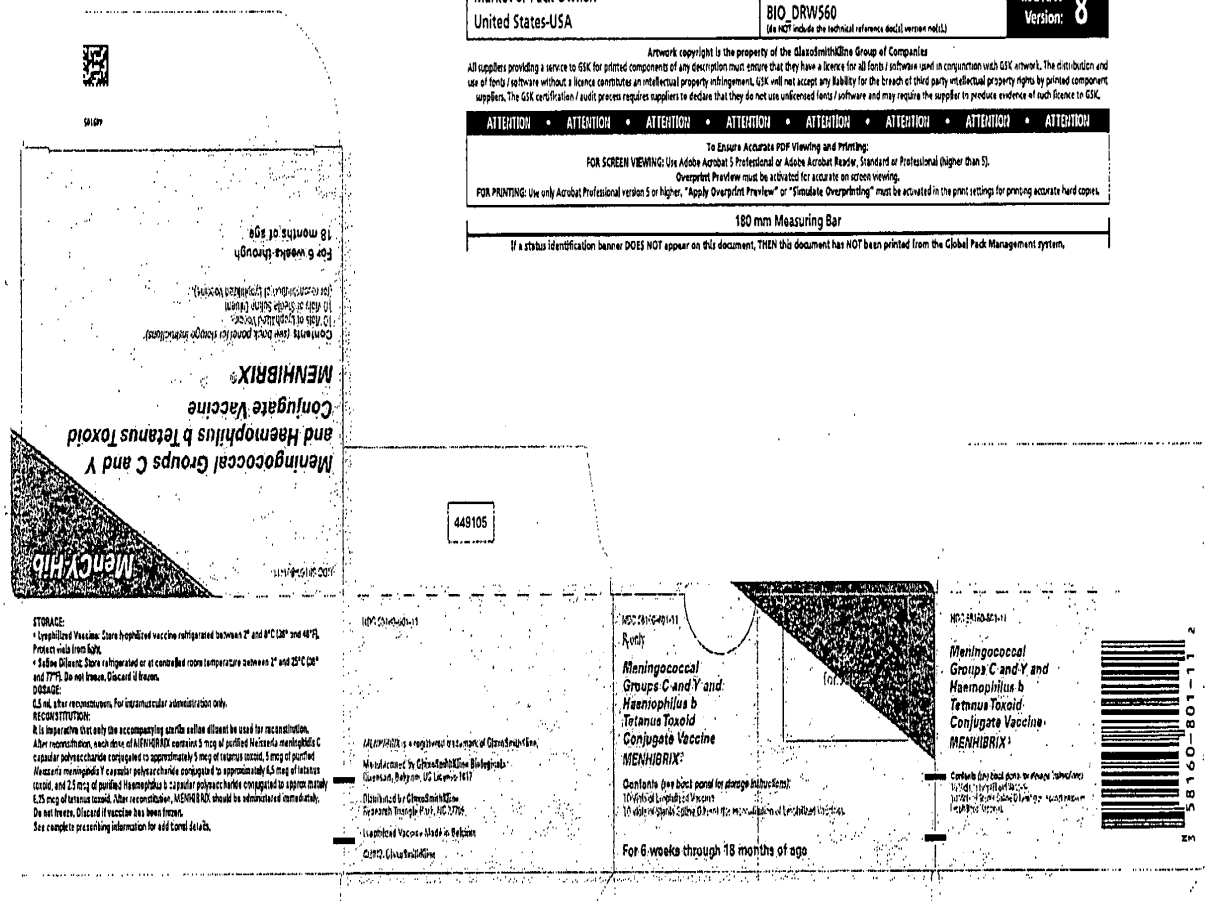

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PAGE 2 OF 2

GlaxoSmithKline Artwork Information Panel	Market Trade Name: Menhibrix	No. of Colours: 3 (does NOT include Varnish, if applicable)
Item Number: 449105	List Colours: (Include sample in fields provided; e.g. 1pet / 1pan-CMYK equivalent)	K · 477
Manufacturing Site: GSK-BEL-Wavre-BEWAV-Marietta		
Market or Pack Owner: United States-USA	Technical Reference No(s).: BIO_DRW560 (do NOT include the technical reference doc(s) version no(s).)	RSC A/W Version: 8

Biologicals Additional Information Panel
Material weight: FAMILY 10
Removable parts n.: N/A
Datamatrix code value: 449105BBN

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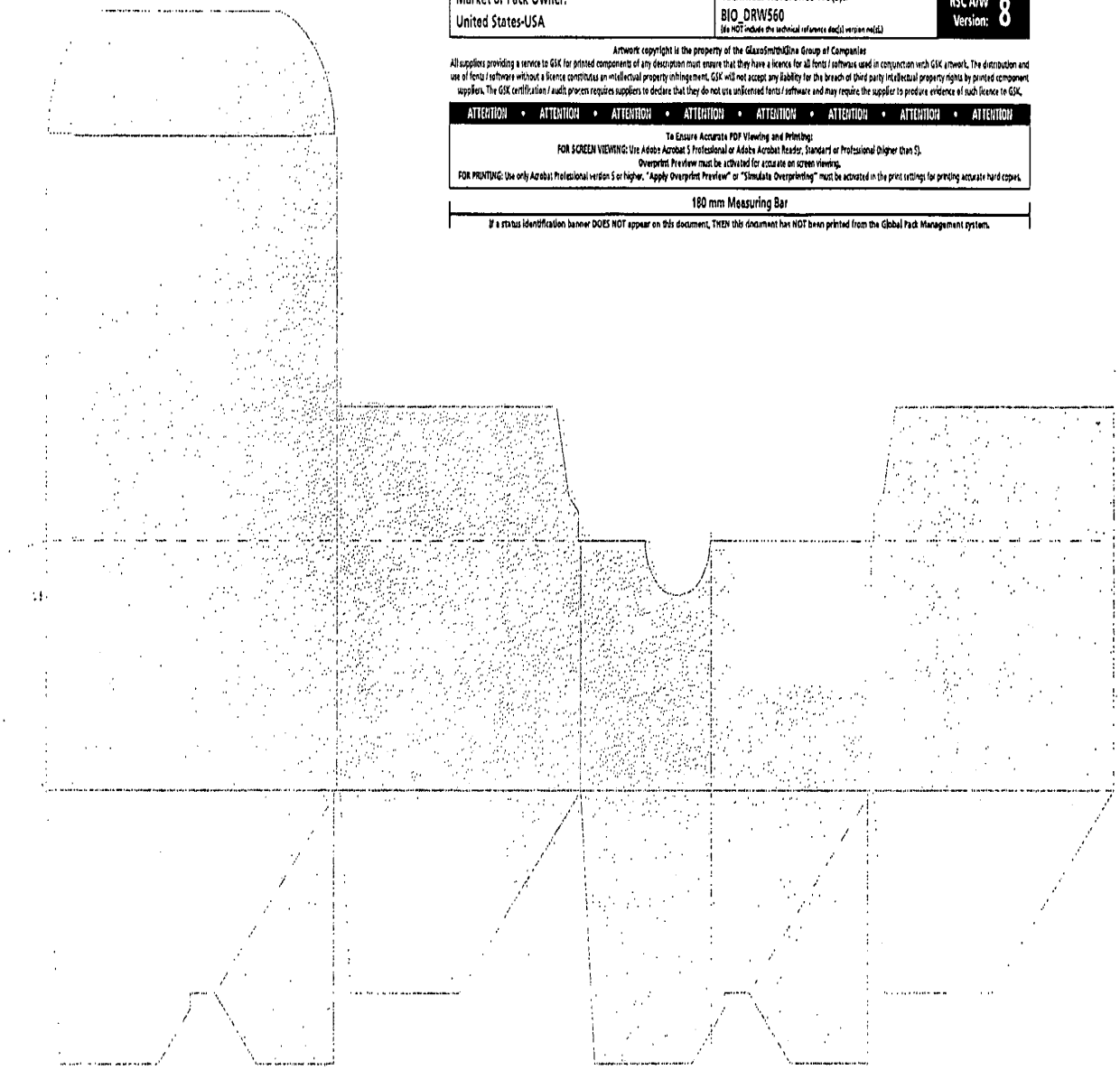

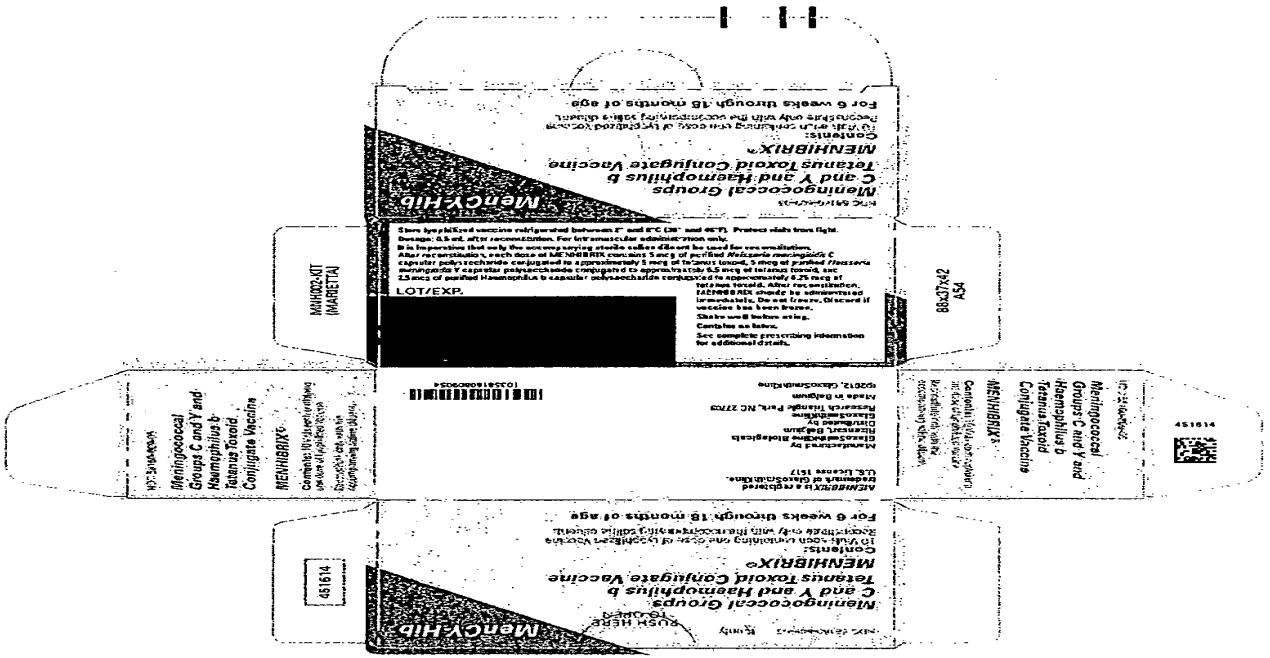

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PAGE 1 OF 2

Biologicals Additional Information Panel
Material weight: FAMILY S
Removable parts n.: N/A
Datamatrix code value: 451614BBO

GlaxoSmithKline Artwork Information Panel	Market Trade Name: Menhibrix	No. of Colours: 3 (does NOT include Varnish, if applicable)
Item Number: 451614	List Colours: (include complete list in Reds provided; e.g. spot / spot-CMYK equivalent)	K, 427
Manufacturing Site: GSK-BEL-Wavre-BEWAV-Marietta		
Market or Pack Owner: United States-USA	Technical Reference No(s).: BIO_DRW533 (do NOT include the technical reference dash(-) version suffix!)	RSC A/W Version: 4

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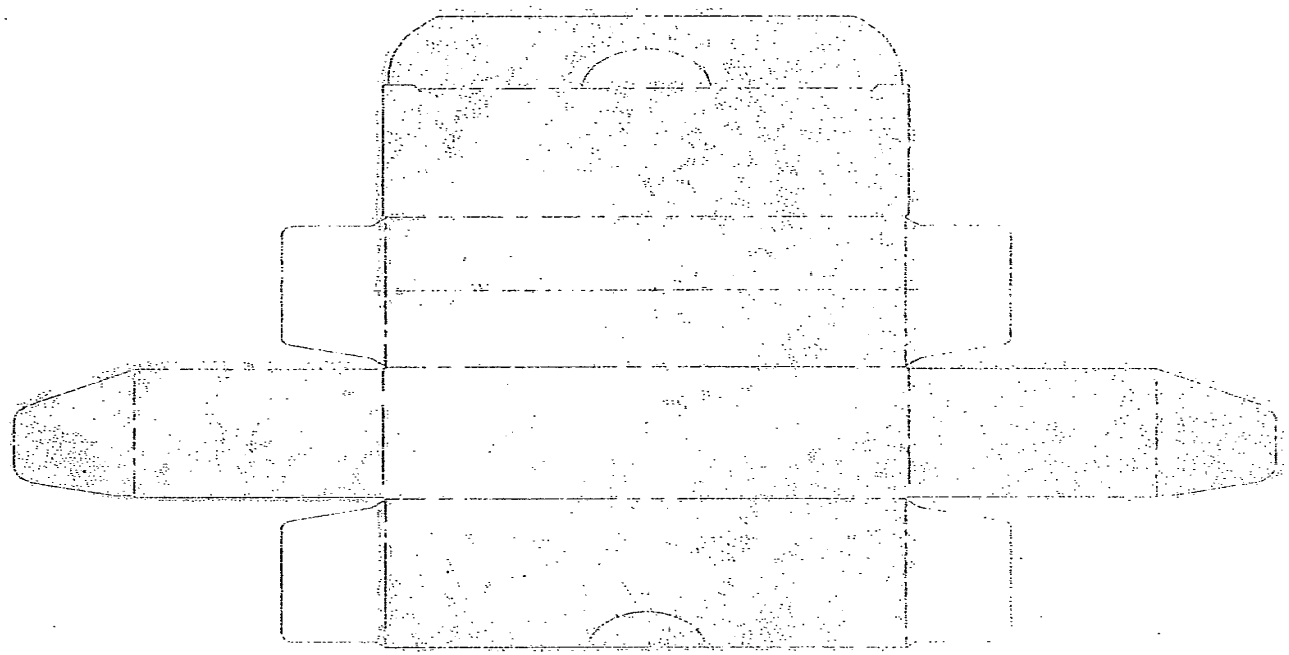

PAGE 2 OF 2

Biologicals Additional Information Panel
Material weight: FAMILY S
Removable parts n.: N/A
Datamatrix code value: 451614BBO

GlaxoSmithKline Artwork Information Panel	Market Trade Name: Menhibrix					No. of Colours: 3 (does NOT include Varnish, if applicable)
Item Number: 451614	List Colours: (Provide sample in Pantone provided; e.g. spot / spot-CMYK equivalent)	K	477			
Manufacturing Site: GSK-BEL-Wavre-BEWAV-Marietta						
Market or Pack Owner: United States-USA	Technical Reference No(s).: BIO_DRW533 (do NOT include the technical reference docId version no(s))					RSC A/W Version: 4

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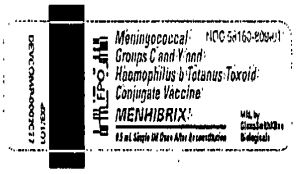

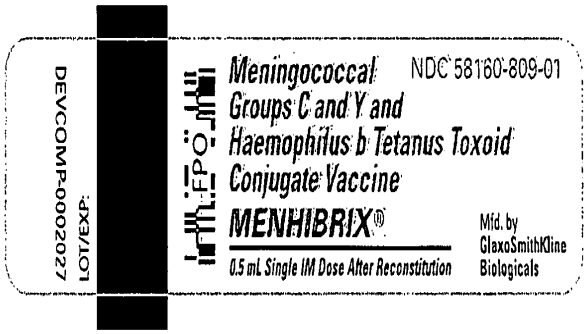

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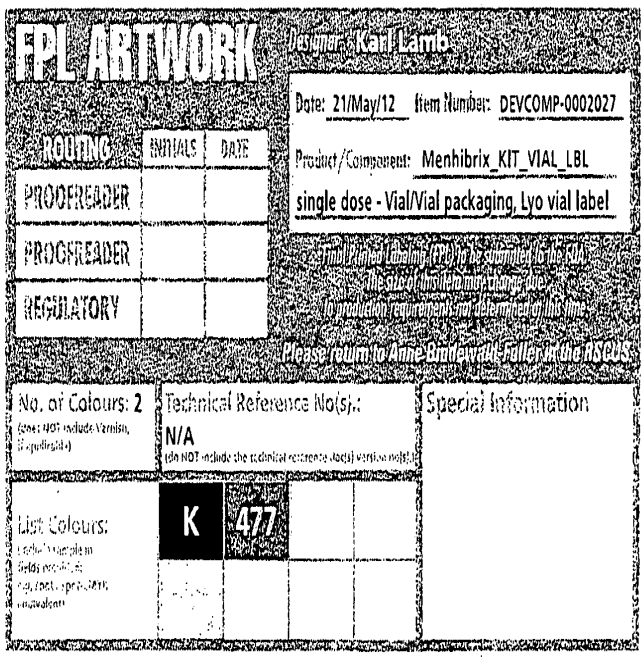

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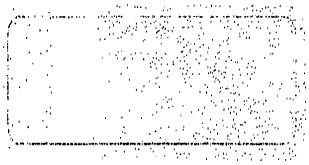

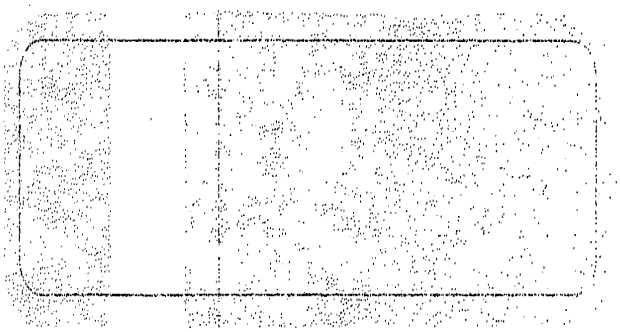

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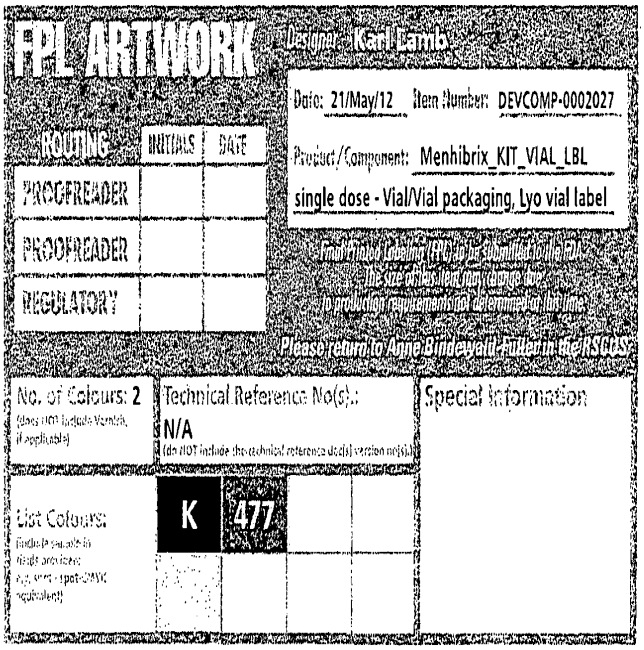

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(Illustrator files workflow)

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use MENHIBRIX safely and effectively. See full prescribing information for MENHIBRIX.

MENHIBRIX (Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine)
Solution for Intramuscular Injection
Initial U.S. Approval: 2012

---------- INDICATIONS AND USAGE ----------

MENHIBRIX is a vaccine indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroups C and Y and *Haemophilus influenzae* type b. MENHIBRIX is approved for use in children 6 weeks of age through 18 months of age. (1)

----------DOSAGE AND ADMINISTRATION----------

Four doses (0.5 mL each) by intramuscular injection at 2, 4, 6, and 12 through 15 months of age. The first dose may be given as early as 6 weeks of age. The fourth dose may be given as late as 18 months of age. (2.3)

----------DOSAGE FORMS AND STRENGTHS----------

Solution for injection supplied as a single-dose vial of lyophilized vaccine to be reconstituted with the accompanying vial of saline diluent. A single dose after reconstitution is 0.5 mL. (3)

---------- CONTRAINDICATIONS ----------

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any meningococcal-, *H. influenzae* type b-, or tetanus toxoid-containing vaccine or any component of MENHIBRIX. (4)

----------WARNINGS AND PRECAUTIONS----------

- If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including MENHIBRIX, should be based on consideration of the potential benefits and possible risks. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including MENHIBRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including MENHIBRIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination. (5.3)

---------- ADVERSE REACTIONS ----------

Rates of local injection site pain, redness, and swelling ranged from 15% to 46% depending on reaction and specific dose in schedule. Commonly reported systemic events included irritability (62% to 71%), drowsiness (49% to 63%), loss of appetite (30% to 34%), and fever (11% to 26%) (specific rate depended on the event and dose in the schedule). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- DRUG INTERACTIONS ----------

Do not mix MENHIBRIX with any other vaccine in the same syringe or vial. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: xx/2012

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2 DOSAGE AND ADMINISTRATION
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  2.2 Administration
  2.3 Dose and Schedule
3 DOSAGE FORMS AND STRENGTHS
4 CONTRAINDICATIONS
5 WARNINGS AND PRECAUTIONS
  5.1 Guillain-Barré Syndrome
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*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

MENHIBRIX® is indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroups C and Y and *Haemophilus influenzae* type b. MENHIBRIX is approved for use in children 6 weeks of age through 18 months of age.

2 DOSAGE AND ADMINISTRATION

2.1 Reconstitution

MENHIBRIX is to be reconstituted only with the accompanying saline diluent. The reconstituted vaccine should be a clear and colorless solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

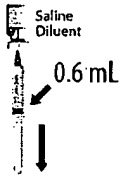

Figure 1. Cleanse both vial stoppers. Withdraw 0.6 mL of saline from diluent vial.

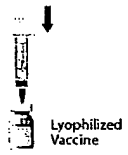

Figure 2. Transfer saline diluent into the lyophilized vaccine vial.

Figure 3. Shake the vial well.

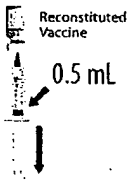

Figure 4. After reconstitution, withdraw 0.5 mL of reconstituted vaccine and administer **intramuscularly**.

2.2 Administration

For intramuscular use only. Do not administer this product intravenously, intradermally, or subcutaneously.

After reconstitution, administer MENHIBRIX immediately.

Use a separate sterile needle and sterile syringe for each individual. The preferred administration site is the anterolateral aspect of the thigh for most infants younger than 1 year of age. In older children, the deltoid muscle is usually large enough for an intramuscular injection.

2.3 Dose and Schedule

A 4-dose series, with each 0.5-mL dose given by intramuscular injection at 2, 4, 6, and 12 through 15 months of age. The first dose may be given as early as 6 weeks of age. The fourth dose may be given as late as 18 months of age.

3 DOSAGE FORMS AND STRENGTHS

MENHIBRIX is a solution for injection supplied as a single-dose vial of lyophilized vaccine to be reconstituted with the accompanying vial of saline diluent. A single dose after reconstitution is 0.5 mL.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any meningococcal-, *H. influenzae* type b-, or tetanus toxoid-containing vaccine or any component of this vaccine is a contraindication to administration of MENHIBRIX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including MENHIBRIX, should be based on consideration of the potential benefits and possible risks.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including MENHIBRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including MENHIBRIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination.

5.4 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the patient's immunization history for possible vaccine hypersensitivity. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

5.5 Altered Immunocompetence

Safety and effectiveness of MENHIBRIX in immunosuppressed children have not been evaluated. If MENHIBRIX is administered to immunosuppressed children, including children receiving immunosuppressive therapy, the expected immune response may not be obtained.

5.6 Tetanus Immunization

Immunization with MENHIBRIX does not substitute for routine tetanus immunization.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the

clinical trials of another vaccine, and may not reflect the rates observed in practice. There is the possibility that broad use of MENHIBRIX could reveal adverse reactions not observed in clinical trials.

A total of 7,521 infants received at least one dose of MENHIBRIX in 6 clinical studies.[1-6] In 5 of these studies, 6,686 children received 4 consecutive doses of MENHIBRIX.[2-6] Across all studies, approximately half of participants were female; 50% were white, 41% were Hispanic, 4% were black, 1% were Asian and 4% were of other racial/ethnic groups.

Two randomized, controlled, pivotal trials enrolled participants to receive 4 doses of MENHIBRIX or a monovalent Haemophilus b Conjugate (Hib) vaccine, administered at 2, 4, 6, and 12 to 15 months of age (Study 009/010[5] and Study 011/012[6]). Together, these trials evaluated safety in 8,571 infants who received at least one dose of MENHIBRIX (N = 6,414) or Hib vaccine (N = 2,157).[5,6]

In Study 009/010[5], conducted in the United States, Australia, and Mexico, 4,180 infants were randomized 3:1 to receive MENHIBRIX or a control US-licensed Hib vaccine. Safety data are available for 3,136 infants who received MENHIBRIX and 1,044 infants who received a control Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate) (PRP-T, manufactured by Sanofi Pasteur SA) at 2, 4, and 6 months of age. For dose 4 administered at 12 to 15 months of age, safety data are available for 2,769 toddlers who received MENHIBRIX and 923 toddlers who received a control Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate) (PRP-OMP, manufactured by Merck and Co., Inc.). With doses 1, 2, and 3 of MENHIBRIX or PRP-T, infants concomitantly received PEDIARIX® [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine] and Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein) (PCV7, manufactured by Wyeth Pharmaceuticals, Inc.). With dose 4 of MENHIBRIX or PRP-OMP, toddlers concomitantly received PCV7, Measles, Mumps, and Rubella Virus Vaccine Live (MMR, manufactured by Merck & Co., Inc.), and Varicella Virus Vaccine Live (manufactured by Merck & Co., Inc.).

Data on solicited adverse events were collected by parents/guardians using standardized forms for 4 consecutive days following vaccination with MENHIBRIX or control Hib vaccine (i.e., day of vaccination and the next 3 days).[5] Children were monitored for unsolicited adverse events that occurred in the 31-day period following vaccination and were monitored for serious adverse events, new onset chronic disease, rash, and conditions prompting emergency department visits or physician office visits during the entire study period (6 months following the last vaccine administered). Among participants in both groups, 66% were from the United States, 19% were from Mexico, and 14% were from Australia. Forty-eight percent of participants were female; 64% were white, 22% were Hispanic, 6% were black, 1% were Asian, and 7% were of other racial/ethnic groups.

In the second pivotal study (Study 011/012[6]), conducted in the United States and Mexico and evaluating the same vaccines and vaccination schedule, participants were monitored for serious adverse events, new onset chronic disease, rash, and conditions prompting emergency

department visits during the entire study period (6 months following the last vaccine administered). Among participants in both groups, 30% were from the United States and 70% were from Mexico.

In addition to the pivotal studies, safety data are available from 4 studies which either did not include a fourth dose of MENHIBRIX[1], used a dosing regimen not approved in the United States[2,3], or incorporated a comparator vaccine which was not licensed in the United States.[4] In these studies, participants were monitored for unsolicited adverse events and serious adverse events occurring in the 31–day period following vaccination. In 2 of these studies,[3,4] participants were monitored for serious adverse events, new onset chronic disease, rash, and conditions prompting emergency department visits or physician office visits through 6 months after the last vaccination.

Solicited Adverse Events: The reported frequencies of solicited local and systemic adverse events from US participants in Study 009/010 are presented in Table 1.[5] Because of differences in reported rates of solicited adverse events between US and non-US participants, only the solicited adverse event data in US participants are presented. Among the US participants included in Table 1, 48% were female; 76% were white, 10% were black, 4% were Hispanic, 2% were Asian, and 8% were of other racial/ethnic groups.

Table 1. Percentage of US Children from Study 009/010 With Solicited Local and General Adverse Events within 4 Days of Vaccination[a] With MENHIBRIX or Haemophilus b Conjugate Vaccine (Total Vaccinated Cohort)

	MENHIBRIX[b]				Haemophilus b Conjugate Vaccine[b,c]			
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 1	Dose 2	Dose 3	Dose 4
Local[d]								
N	2,009	1,874	1,725	1,533	659	612	569	492
Pain, any	46.2	44.6	41.4	42.1	61.6	52.8	49.9	50.4
Pain, grade 3[e]	3.7	3.3	2.3	1.6	11.4	5.1	3.0	5.3
Redness, any	20.6	31.0	35.5	34.6	27.9	33.7	42.2	46.7
Redness, >30 mm	0.1	0.3	0.1	0.7	1.8	0.3	0.4	1.2
Swelling, any	14.7	20.4	23.8	25.4	20.5	20.8	28.6	31.7
Swelling, >30 mm	0.5	0.3	0.3	0.6	1.5	0.2	0.4	0.8
Systemic								
N	2,008-2,009	1,871	1,723	1,535-1,536	659	609-610	569	493-494
Irritability	67.5	70.8	65.8	62.1	76.9	75.1	65.4	66.1
Irritability, grade 3[f]	3.7	4.8	3.3	2.5	7.4	5.6	4.2	4.3
Drowsiness, any	62.8	57.7	49.5	48.7	66.9	61.8	52.4	48.5
Drowsiness, grade 3[g]	2.7	3.2	1.7	2.1	2.7	2.6	1.4	2.0
Loss of appetite, any	33.8	32.1	30.1	32.1	37.6	33.6	30.2	32.5
Loss of appetite, grade 3[h]	0.5	0.7	0.5	1.1	0.3	0.7	1.1	2.2
Fever, ≥100.4°F[i]	18.9	25.9	23.0	11.0	21.4	28.2	23.7	12.6
Fever, ≥102.2°F[i]	1.1	1.9	3.2	1.5	0.9	2.6	2.8	2.0
Fever, >104°F[i]	0.0	0.1	0.3	0.3	0.0	0.0	0.4	0.2

Total Vaccinated Cohort = all participants who received at least one dose of either vaccine.

N = number of participants who completed the symptom sheet for a given symptom at the specified dose.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] Co-administered with PEDIARIX and PCV7 at doses 1, 2, 3 and PCV7, MMR and varicella vaccines at dose 4.

[c] US-licensed monovalent Haemophilus b Conjugate Vaccine manufactured by Sanofi Pasteur SA for doses 1, 2, and 3 (PRP-T) and by Merck & Co., Inc for dose 4 (PRP-OMP).

[d] Local reactions at the injection site for MENHIBRIX or Haemophilus b Conjugate Vaccine.

[e] Cried when limb was moved/spontaneously painful.

[f] Crying that could not be comforted/prevented normal daily activities.

[g] Prevented normal daily activities.

[h] Not eating at all.

[i] Across both treatment groups, 54%, 56%, and 59% of participants had temperatures measured rectally following doses 1, 2, and 3, respectively; 45%, 44%, and 40% of participants had temperatures measured by the axillary route for doses 1, 2, and3, respectively. For dose 4, >90% of participants had temperatures measured via the axillary route.

The reported rates of some solicited adverse events in participants from Australia and Mexico varied from those in the United States.[5] For example, in Australia, pain after dose 1 was reported in 28.4% of participants who received MENHIBRIX and 33.3% of control participants, while in Mexico pain after dose 1 was reported in 73.7% of participants who received MENHIBRIX and 79.4% of control participants. Fever after dose 1 was reported in 10.4% of participants who received MENHIBRIX and 10.7% of control participants in Australia, while it was reported in 44.0% of participants who received MENHIBRIX and 35.7% of control participants in Mexico. The reported incidences of pain and fever in US participants after dose 1 are provided in Table 1.

Unsolicited Adverse Events: Among participants who received MENHIBRIX or Hib control vaccine co-administered with US-licensed vaccines at 2, 4, 6 and 12 to 15 months of age[1,3-5], the incidence of unsolicited adverse events reported within the 31-day period following study vaccination (doses 1, 2, and 3) was comparable between MENHIBRIX (61.9%; 2,578/4,166) and PRP-T (62.5%; 1,042/1,666). The incidence of unsolicited adverse events reported within the 31-day period following dose 4 was also comparable between MENHIBRIX (42.5%; 1,541/3,630) and PRP-OMP (41.4%; 520/1,257).

Serious Adverse Events: Following doses 1, 2, and 3[1,3-6], 1.8% (137/7,444) of participants who received MENHIBRIX and 2.1% (59/2,779) of participants who received PRP-T group reported at least one serious adverse event within the 31-day period. Up to 6 months following the last vaccine administered (doses 1, 2, and 3) or until administration of dose 4[3-6], 4.8% (365/7,362) of participants who received MENHIBRIX and 5.0% (134/2,697) of participants in the PRP-T group reported at least one serious adverse event.

Following dose 4[3-6], 0.5% (35/6,640) of participants who received MENHIBRIX and 0.5% (12/2,267) of participants who received PRP-OMP reported at least one serious adverse event within the 31-day period. Up to 6 months following the last vaccine administered (dose 4), 2.5% (165/6,640) of participants who received MENHIBRIX and 2.0% (46/2,267) of participants who received PRP-OMP reported at least one serious adverse event.

6.2 Postmarketing Experience

The following adverse events have been spontaneously reported during post-approval use of HIBERIX® (Haemophilus b Conjugate Vaccine [Tetanus Toxoid Conjugate]) in the United States and other countries. These events are relevant because the Haemophilus b capsular polysaccharide tetanus toxoid conjugate is included as a component antigen in both MENHIBRIX and HIBERIX. Because these events are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or to establish a causal relationship to vaccine exposure.

The following adverse events were included based on one or more of the following factors: seriousness, frequency of reporting, or strength of evidence for a causal relationship to HIBERIX.

General Disorders and Administration Site Conditions: Extensive swelling of the vaccinated limb, injection site induration.

Immune System Disorders: Allergic reactions (including anaphylactic and anaphylactoid reactions), angioedema.

Nervous System Disorders: Convulsions (with or without fever), hypotonic-hyporesponsive episode, somnolence, syncope or vasovagal responses to injection.

Respiratory, Thoracic, and Mediastinal Disorders: Apnea.

Skin and Subcutaneous Tissue Disorders: Rash, urticaria.

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

In clinical studies, MENHIBRIX was administered concomitantly with routinely recommended pediatric US-licensed vaccines *[see Adverse Reactions (6.1) and Clinical Studies (14.2)]*.

If MENHIBRIX is administered concomitantly with other injectable vaccines, they should be given with separate syringes and at different injection sites. MENHIBRIX should not be mixed with any other vaccine in the same syringe or vial.

7.2 Interference With Laboratory Tests

Haemophilus b capsular polysaccharide derived from Haemophilus b Conjugate Vaccines has been detected in the urine of some vaccinees.[7] Urine antigen detection may not have a diagnostic value in suspected disease due to *H. influenzae* type b within 1 to 2 weeks after receipt of a *H. influenzae* type b-containing vaccine, including MENHIBRIX.

7.3 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to MENHIBRIX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Animal reproduction studies have not been conducted with MENHIBRIX. It is also not known whether MENHIBRIX can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity.

8.4 Pediatric Use

Safety and effectiveness of MENHIBRIX in children younger than 6 weeks of age and in children 19 months to 16 years of age have not been established.

11 DESCRIPTION

MENHIBRIX (Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine), for intramuscular injection, is supplied as a sterile, lyophilized powder which is reconstituted at the time of use with the accompanying saline diluent. MENHIBRIX contains *Neisseria meningitidis* serogroup C and Y capsular polysaccharide antigens and Haemophilus b capsular polysaccharide (polyribosyl-ribitol-phosphate [PRP]). The *Neisseria meningitidis* C strain and Y strain are grown in semi-synthetic media and undergo heat inactivation and purification. The PRP is a high molecular weight polymer prepared from the *Haemophilus influenzae* type b strain 20,752 grown in a synthetic medium that undergoes heat inactivation and purification. The tetanus toxin, prepared from *Clostridium tetani* grown in a semi-synthetic medium, is detoxified with formaldehyde and purified. Each capsular polysaccharide is individually covalently bound to the inactivated tetanus toxoid. After purification, the conjugate is lyophilized in the presence of sucrose as a stabilizer. The diluent for MENHIBRIX is a sterile saline solution (0.9% sodium chloride) supplied in vials.

When MENHIBRIX is reconstituted with the accompanying saline diluent, each 0.5-mL dose is formulated to contain 5 mcg of purified *Neisseria meningitidis* C capsular polysaccharide conjugated to approximately 5 mcg of tetanus toxoid, 5 mcg of purified *Neisseria meningitidis* Y capsular polysaccharide conjugated to approximately 6.5 mcg of tetanus toxoid, and 2.5 mcg of purified Haemophilus b capsular polysaccharide conjugated to approximately 6.25 mcg of tetanus toxoid. Each dose also contains 96.8 mcg of Tris (trometamol)-HCl, 12.6 mg of sucrose, and ≤0.72 mcg of residual formaldehyde. MENHIBRIX does not contain preservatives. The vial stoppers do not contain latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Neisseria meningitidis: The presence of bactericidal anti-capsular meningococcal antibodies has been associated with protection from invasive meningococcal disease.[8] MENHIBRIX induces production of bactericidal antibodies specific to the capsular polysaccharides of serogroups C and Y.

Haemophilus influenzae type b: Specific levels of antibodies to PRP (anti-PRP) have been shown to correlate with protection against invasive disease due to *H. influenzae* type b. Based on data from passive antibody studies[9] and a clinical efficacy study with unconjugated *Haemophilus* b polysaccharide vaccine[10], an anti-PRP concentration of 0.15 mcg/mL has been accepted as a minimal protective level. Data from an efficacy study with unconjugated *Haemophilus* b polysaccharide vaccine indicate that an anti-PRP concentration of ≥1.0 mcg/mL predicts protection through at least a 1-year period.[11,12] These antibody levels have been used to evaluate the effectiveness of *H. influenzae* type b-containing vaccines, including MENHIBRIX.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

MENHIBRIX has not been evaluated for carcinogenic or mutagenic potential, or for impairment of fertility.

14 CLINICAL STUDIES

14.1 Immunological Evaluation

In Study 009/010[5] the immune response to MENHIBRIX and control vaccines was evaluated in a subset of US participants. In this clinical study, MENHIBRIX and Hib control vaccines were administered concomitantly with routinely recommended US-licensed vaccines *[see Adverse Reactions (6.1)]*. Among participants in the ATP immunogenicity cohort for both vaccine groups combined, 47% were female; 81% of participants were white, 8% were black, 4% were Hispanic, 1% were Asian, and 6% were of other racial/ethnic groups.

Study objectives included evaluation of *N. meningitidis* serogroups C (MenC) and Y (MenY) as measured by serum bactericidal assay using human complement (hSBA) and antibodies to PRP as measured by enzyme-linked immunosorbent assay (ELISA) in sera obtained approximately one month (range 21 to 48 days) after dose 3 of MENHIBRIX or PRP-T and approximately 6 weeks (range 35 to 56 days) after dose 4 of MENHIBRIX or PRP-OMP. The hSBA-MenC and hSBA-MenY geometric mean antibody titers (GMTs) and the percentage of participants with hSBA-MenC and hSBA-MenY levels ≥1:8 are presented in Table 2. Anti-PRP geometric mean antibody concentrations (GMCs) and the percentage of participants with anti-PRP levels ≥0.15 mcg/mL and ≥1.0 mcg/mL are presented in Table 3.

Table 2. Bactericidal Antibody Responses Following MENHIBRIX (One Month After Dose 3 and 6 Weeks After Dose 4) in US Children Vaccinated at 2, 4, 6, and 12 to 15 Months of Age (ATP Cohort for Immunogenicity)

	MENHIBRIX Post-Dose 3	MENHIBRIX Post-Dose 4
hSBA-MenC	N = 491	N = 331
% ≥1:8	98.8	98.5[a]
95% CI	97.4, 99.6	96.5, 99.5
GMT	968	2040
95% CI	864, 1084	1746, 2383
hSBA-MenY	N = 481	N = 342
% ≥1:8	95.8	98.8[a]
95% CI	93.7, 97.4	97.0, 99.7
GMT	237	1390
95% CI	206, 272	1205, 1602

ATP = according to protocol; CI = confidence interval; GMT = geometric mean antibody titer.
N = number of US children eligible for inclusion in the ATP immunogenicity cohort for whom serological results were available for the post-dose 3 and post-dose 4 immunological evaluations.
[a] Acceptance criteria were met (lower limit of 95% CI for the percentage of participants with hSBA-MenC and hSBA-MenY titers ≥1:8 ≥90% following 4 doses).

Table 3. Comparison of anti-PRP Responses Following MENHIBRIX or Haemophilus b Conjugate Vaccine[a] (One Month After Dose 3 and 6 Weeks After Dose 4) in US Children Vaccinated at 2, 4, 6, and 12 to 15 Months of Age (ATP Cohort for Immunogenicity)

	Post-Dose 3		Post-Dose 4	
	MENHIBRIX	**PRP-T**	**MENHIBRIX**	**PRP-OMP**
Anti-PRP	N = 518	N = 171	N = 361	N = 126
% ≥0.15 mcg/mL	100	98.2	100	100
95% CI	99.3, 100	95.0, 99.6	99.0, 100	97.1, 100
% ≥1.0 mcg/mL	96.3[b]	91.2	99.2[b]	99.2
95% CI	94.3, 97.8	85.9, 95.0	97.6, 99.8	95.7, 100
GMC (mcg/mL)	11.0	6.5	34.9	20.2
95% CI	10.0, 12.1	5.3, 7.9	30.7, 39.6	16.4, 24.9

ATP = according to protocol; anti-PRP = antibody concentrations to *H. influenzae* capsular polysaccharide; CI = confidence interval; GMC = geometric mean antibody concentration.
N = number of US children eligible for inclusion in the ATP immunogenicity cohort for whom serological results were available for the post-dose 3 and post-dose 4 immunological evaluations.

[a] US-licensed monovalent Haemophilus b Conjugate Vaccine for doses 1, 2, and 3 (PRP-T) and for dose 4 (PRP-OMP).

[b] Non-inferiority was demonstrated (lower limit of 95% CI on the group difference of MENHIBRIX minus Haemophilus b Conjugate Vaccine ≥-10%).

14.2 Concomitant Vaccine Administration

In participants who received MENHIBRIX concomitantly with PEDIARIX and PCV7 at 2, 4, and 6 months of age, there was no evidence for reduced antibody response to pertussis antigens (GMC to pertussis toxin, filamentous hemagglutinin, and pertactin), diphtheria toxoid (antibody levels ≥0.1 IU/mL), tetanus toxoid (antibody levels ≥0.1 IU/mL), poliovirus types 1, 2, and 3 (neutralizing antibody levels ≥1:8 to each virus), hepatitis B (anti-hepatitis B surface antigen ≥10 mIU/mL) or PCV7 (antibody levels ≥0.2 mcg/mL and GMC to each serotype) relative to the response in control participants administered PRP-T concomitantly with PEDIARIX and PCV7. The immune responses to PEDIARIX[3,5] and PCV7[3] were evaluated one month following dose 3.

There was no evidence for interference in the immune response to MMR and varicella vaccines (initially seronegative participants with anti-measles ≥200 mIU/mL, anti-mumps ≥51 ED_{50}, anti-rubella ≥10 IU/mL, and anti-varicella ≥1:40) administered at 12 to 15 months of age concomitantly with MENHIBRIX and PCV7 relative to these vaccines administered concomitantly with PRP-OMP and PCV7.[4,5] The immune responses to MMR and varicella vaccines were evaluated 6 weeks post-vaccination. Data are insufficient to evaluate potential interference when a fourth PCV7 dose is administered concomitantly with MENHIBRIX at 12 to 15 months of age.

15 REFERENCES

All NCT numbers are as noted in the National Library of Medicine clinical trial database (see www.clinicaltrials.gov).

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11. Käythy H, Peltola H, Karanko V, et al. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1983;147:1100.
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16 HOW SUPPLIED/STORAGE AND HANDLING

MENHIBRIX is available in single-dose vials of lyophilized vaccine, accompanied by vials containing 0.85 mL of saline diluent (packaged without syringes or needles).

Supplied as package of 10 doses (NDC 58160-801-11):

NDC 58160-809-01 Vial of lyophilized vaccine in Package of 10: NDC 58160-809-05

NDC 58160-813-01 Vial of saline diluent in Package of 10: NDC 58160-813-05

16.1 Storage Before Reconstitution

Lyophilized vaccine vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light.

Diluent: Store refrigerated or at controlled room temperature between 2° and 25°C (36° and 77°F). Do not freeze. Discard if the diluent has been frozen.

16.2 Storage After Reconstitution

After reconstitution, administer MENHIBRIX immediately. Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

- Inform parents or guardians of the potential benefits and risks of immunization with MENHIBRIX, and of the importance of completing the immunization series.
- Inform parents or guardians about the potential for adverse reactions that have been temporally associated with administration of MENHIBRIX or other vaccines containing similar components.
- Instruct parents or guardians to report any adverse events to their healthcare provider.
- Give parents or guardians the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

HIBERIX, MENHIBRIX, and PEDIARIX are registered trademarks of GlaxoSmithKline.

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Research Triangle Park, NC 27709

MNX:1PI

Exhibit F

Copy of the terminal disclaimer, certificate of correction, certificate for correction of inventorship, and receipt showing payment of the maintenance fees for the '326 patent

PATENT
Attorney Docket No. 04995.0005-02000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Andrew Lees	)	
	)	
Serial No.: 08/456,694	)	Group Art Unit: 1818
	)	
Filed: June 1, 1995	)	Examiner: P. Achutamurthy

For: PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS

TERMINAL DISCLAIMER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Petitioner ("Assignee"), Henry M. Jackson Foundation for the Advancement of Military Medicine, whose post office address is 1401 Rockville Pike, Suite 600, Rockville, Maryland, 20852, represents that it is the only Assignee of the entire right, title, and interest in and to both the above-identified patent application, U.S. Patent Application No. 08/456,694, filed June 1, 1995, for PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC CYANYLATING REAGENTS, in the name of Andrew Lees, as indicated by the Assignment filed for recordation in the United States Patent and Trademark Office on April 11, 1997 (copy attached), and is the only Assignee of U.S. Patent Application No. 08/408,717, filed March 22, 1995, for PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED VIA ORGANIC

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WASHINGTON, D. C. 20005
202-408-4000

CYANYLATING REAGENTS, in the name of Andrew Lees, as indicated by the Assignment filed for recordation in the United States Patent and Trademark Office on April 11, 1997. It is noted that U.S. Patent Application No. 08/456,694, filed June 1, 1995, is a continuation of U.S. Patent Application No. 08/408,717, filed March 22, 1995, and therefore, a single Assignment, filed in U.S. Patent Appl. No. 08/408,717, transfers the inventor's right, title, and interest in and to both applications to the Petitioner.

To obviate a double patenting rejection, Henry M. Jackson Foundation for the Advancement of Military Medicine, through its undersigned attorney of record, hereby disclaims, under the provisions of 37 C.F.R. § 1.321, the terminal part of any patent granted on U.S. Patent Application No. 08/456,694 which would extend beyond the expiration date of any patent granted on U.S. Patent Application No. 08/408,717, and hereby agrees that any patent so granted on U.S. Patent Application No. 08/456,694 shall be enforceable only for and during such period that the legal title to said patent shall be the same as the legal title to any patent granted on U.S. Patent Application No. 08/408,717, this agreement to run with any patent granted on U.S. Patent Application No. 08/456,694, and to be binding upon the grantee, its successors, or assigns.

In making the above disclaimer, Petitioner does not disclaim the terminal part of any patent granted on U.S. Patent Application No. 08/456,694 that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. §§ 154 to 156 and 173 of any patent granted on U.S. Patent Application No. 08/408,717 in the event that any such granted patent on

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U.S. Patent Application No. 08/408,717 expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or in part, is terminally disclaimed under 37 C.F.R. § 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term.

In accordance with the fee schedule set forth in 37 C.F.R. § 1.20(d), the required fee of $110.00 is filed with this disclaimer.

If a check for the required fee is not filed concurrently herewith or if there are any additional fees due in connection with the filing of this Terminal Disclaimer, please charge the fees to our Deposit Account No. 06-0916. If a fee is required for an extension of time under 37 C.F.R. § 1.136 that is not accounted for, such an extension is requested, and the fee also should be charged to Deposit Account 06-0916. Any overpayment may be credited to Deposit Account No. 06-0916.

The undersigned attorney of record is authorized to act on behalf of Assignee, Henry M. Jackson Foundation for the Advancement of Military Medicine.

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

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Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Jean B. Fordis
Jean B. Fordis
PTO Registration No. 32,984

Date: April 11, 1997

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.: 5,693,326

DATED: December 2, 1997

INVENTOR(S): Lees et al.

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

CLAIM 13, Col. 30, L. 61 change "by;" to --by:--.

Face of the patent, change "The portion of the term of this patent subsequent to Mar. 22, 2012, has been disclaimed" to - -The portion of the term of this patent subsequent to the expiration date of U.S. Patent No. 5,651,971 has been disclaimed- -.

Signed and Sealed this

Twenty-fourth Day of October, 2000

Attest:

Michelle Williams

Attesting Officer

Q. TODD DICKINSON

Director of Patents and Trademarks

UNITED STATES PATENT AND TRADEMARK OFFICE

Certificate

Patent No. 5,693,326 Patented: December 2, 1997

On petition requesting issuance of a certificate for correction of inventorship pursuant to 35 U.S.C. 256, it has been found that the above identified patent, through error and without any deceptive intent, improperly sets forth the inventorship.

Accordingly, it is hereby certified that the correct inventorship of this patent is: Andrew Lees, Silver Spring, MD; Clifford M. Snapper, Potomac, MD; and James J. Mond, Silver Spring, MD.

Signed and Sealed this Fifth Day of September, 2000.

PONNATHAPURA ACHUTAMURTHY, *SPE*
Art Unit 1652

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Issue Date:	12/02/1997		Filing Date:	06/01/1995	
Title:	PRODUCING IMMUNOGENIC CONSTRUCTS USING SOLUBLE CARBOHYDRATES ACTIVATED				
Status:	4th, 8th and 12th year fees paid			Entity:	Large
Window Opens:	N/A	Surcharge Date:	N/A	Expiration:	N/A
Fee Amt Due:	Window not open	Surchg Amt Due:	Window not open	Total Amt Due:	Window not open
Fee Code:					
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PATENT NUMBER	FEE AMT	SUR-CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
5,693,326	$4,110.00	$0.00	06/02/09	08/456,694	12/02/97	06/01/95	12	NO	HMJF HJF 008-93 (02)

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Exhibit G

Letter dated May 12, 2004, submitting BB-IND No. 11,706

May 12, 2004

William M. Egan, Ph.D., Acting Director
Office of Vaccines Research and Review
Center for Biologics Evaluation and Research
Attn: Document Control Room, Suite 200N HFM-99
Food and Drug Administration
Woodmont Office Center
1401 Rockville Pike
Rockville, MD 20852-1448

Attn: Dr. Martha Monser, Division of Vaccines and Related Products Applications, HFM-475

Re: BBIND 0; Haemophilus influenzae type b-Neisseria meningitidis serogroup C and Y conjugate vaccine with tetanus toxoid as protein carrier (Hib-MenCY)
Initial Investigational New Drug Application
Serial No.: 0000

Dear Dr. Egan:

Pursuant to 21 CFR 312.20, please find enclosed a GlaxoSmithKline (GSK) sponsored Investigational New Drug Application (IND) to provide for the clinical study of our candidate *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroups C and Y vaccine (tetanus toxoid conjugate) combined, hereto referred to as Hib-MenCY-TT. The proposed indication is for infants/children from 6 weeks to 24 months of age with the vaccine being administered as a 3-dose primary vaccination series with a single booster dose given from 12 to 15 months of age.

Enclosed in this IND is all the necessary information as per 21 CFR 312.20, including the final clinical protocol for our proposed US Phase II study, referred to as Hib-MenCY-TT-005 (primary, 101858) and 006 (booster, 102015) and entitled:

> *"A phase II, single-blinded, randomized, controlled, multicenter primary and booster vaccination study to evaluate immunogenicity, reactogenicity and safety of GSK Biological's Haemophilus influenzae type b and Neisseria meningitidis serogroups C and Y-tetanus toxoid conjugate vaccine combined (Hib-MenCY-TT) compared to ActHIB, each co-administered with Pediarix and Prevnar, in healthy infants at 2, 4 and 6 months of age and in healthy toddlers at 12 to 15 months of age (booster dose), when co-administered with Prenvar. An exploratory control group will receive licensed Menomune at 3 to 5 years of age."*

Reference is made to our pre-IND meeting with the Agency of April 9, 2003. The FDA and GSK minutes are enclosed in Item 10 of this application. As per feedback received and agreements made during that meeting, please note that the following information relevant to opening of the IND and initiation of the US Phase II study can be found in this application as such:

Recommendation/Agreement	IND Section including information
Full study report – German MenC-TT Phase II study (711202/001)	Item 9
Summary of grade 3 local and systemic reactions from MenC-TT 001 (solicited and unsolicited)	Item 9, Section 3.2.1.1 Tables 27-29
Interim study report – Australian Hib-MenCY-TT Phase II study (792014/001 & 002) and number of evaluable subjects in each arm	Item 9
US Phase II Study (Hib-MenCY-TT-005; 10185 & -006; 102015): • Combined primary & booster phases • Comparative immunogenicity for concomitant vaccines & number of evaluable subjects • Sample size to maintain 80% power with 1-sided alpha of 0.025 • Methods for tracking/documenting extensive local reactions (i.e. swelling) • Inclusion of rash in 6 mo. follow-up • Antipyretics/analgesics strongly discouraged • Rescue plan for inadequate response (PRP) • Addition of evaluation of % responders with antibody levels ≥0.5 mcg/ml for Prevnar serotypes • Inclusion of exploratory Menomune arm in US Phase II study (3-5 year olds)	Item 4
Rationale for booster dose of Hib-MenCY-TT	Item 4, Section 6.
Complete information regarding the manufacture and testing of the vaccine (including country of origin for all animal sources)	Item 7
Information on the validation status of the analytical methods used for release of drug substance and drug product	Being compiled, will be submitted as soon as available
Informed consent regarding materials of bovine origin used in manufacture (Phase II study)	Item 4, US Phase II protocol

Please note that in addition to the 2 Phase II studies discussed and requested by FDA at the pre-IND meeting (German MenC-TT/001 & Australian Hib-MenCY-TT-001/2), we have also included in this IND (see Item 9) a statistical report from a third Phase II study conducted in Belgium and Germany (Hib-MenCY-TT-003) with the same 3 formulations of the candidate Hib-MenCY-TT vaccine as used in Australian study Hib-MenCY-TT-001/2.

Please also note that GSK intends to use a new technology, Luminex® Corporation xMAP® technology, in place of the ELISA for the analysis of serum samples for the diphtheria, tetanus and pertussis antigens (see Item 4, Section 7). Additional information regarding this technology will be provided as an amendment to the IND. GSK would greatly appreciate a discussion with the Agency regarding this technology prior to its use in analyzing sera for the Phase II study.

GSK would also like to provide advance notice that we plan to submit an application for Fast Track Designation, as per *Guidance for Industry – Fast Track Drug Development Programs – Designation, Development and Application Review (1998).* An amendment to the IND, which will include the Fast Track application and the comprehensive proposal for the clinical development plan and timings, will be submitted to the IND within the next 2 to 3 months. GSK's rationale for submission of a Fast Track application is several fold and is based on the fact that there is no prophylactic vaccine currently available for the infant population (<2 years of age) for meningococcal disease. Furthermore, meningococcal disease caused by *Neisseria meningitidis* remains a major cause of death and morbidity throughout the world, with an annual attack rate of 1 to 3/100,000 and a fatality rate of 5% in children under 5 years of age in the US population. Although the outcome has improved with the introduction of antibiotics, the disease presentation is often fulminant with no time for antibiotics to be effective, and permanent neurological damage and death can occur after infection despite antibiotic therapy.

It is important to note that GSK feels the available data from our Phase II studies are rather compelling with respect to immunogenicity. These data suggest there is no immunologic interference between components within the vaccine or with components of US-licensed co-administered vaccines. Also important is the fact that this vaccine includes the Hib component to be given on the currently recommended immunization schedule; therefore, no increase in the number of injections currently recommended in the first year of life will be necessary for inclusion of a new and potentially life-saving immunization. It is for these reasons, to be detailed in the amendment, that GSK believes the Hib-MenCY-TT vaccine is a candidate for Fast Track designation.

Please note, most of the literature references in support of this IND have been provided in the application. For those references not provided, copies will be made available upon request. As per the discussions at the pre-IND meeting, this IND is being submitted in fully electronic format. Enclosed you will find 2 copies of the CD-ROM along with the original signed versions of the cover letter and Form FDA 1571 (1/03). The CD-ROM contains a submission roadmap (roadmap.pdf) and electronic navigation (amendtoc.pdf).

We look forward to acceptance of this IND and initiation of the US Phase II study following the mandatory 30-day time period. In the interim, if you have any questions or

need additional information, please contact me by phone at 1-610-787-3762 or by facsimile at 1-610-787-7063.

Sincerely,

Amy Scott-Billman, M.S.
Executive Director
Adult (Acting) & Pediatric Vaccines
US Regulatory Affairs

Exhibit H

Letter from FDA acknowledging May 12, 2004 submission of IND 11,706

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

MAY 2 7 2004

Our Reference: BB-IND 11706

Division of Vaccines and
Related Products Applications
Telephone: (301) 827-3070

GlaxoSmithKline Biologicals SA
Attention: Ms. Amy M. Scott-Billman
US Regulatory Affairs
2301 Renaissance Boulevard
Building 510
P.O. Box 61540
King of Prussia, PA 19406-2772

Dear Ms. Scott-Billman:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND#: **11706**

SPONSOR: **GlaxoSmithKline Biologicals SA**

PRODUCT NAME: ***Haemophilus influenzae* type B (Hib) and *Neisseria meningitidis* Serogroups C and Y – Tetanus Toxoid Conjugate Vaccine (Hib-MenCY-TT)**

DATE OF SUBMISSION: **May 12, 2004**

DATE OF RECEIPT: **May 14, 2004**

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file, including the Form FDA 1571**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above, unless a waiver has been requested and granted. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the

deficiencies is satisfactory. If such a clinical hold is placed on this file you will be notified in writing of the reasons for placing the IND on hold.
You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Annual Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect. Any unexpected fatal or immediately life-threatening reaction which is associated with use of this product must be reported to this Center within seven calendar days, and all serious, unexpected adverse experiences must be reported, in writing, to this Center and to all study centers within fifteen calendar days.

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 12 (7) of Form FDA 1571 requests that either an environmental assessment, or a claim for categorical exclusion from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one.

Sponsors of INDs for products used to treat life-threatening or severely-debilitating illnesses are encouraged to consider the interim rule outlined in 21 CFR 312.80 through 312.88.

Telephone inquiries concerning this IND should be made directly to this Division at (301) 827-3070. Correspondence regarding this file should be addressed as follows:

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Vaccines Research and Review
Division of Vaccines and Related Products Applications
1401 Rockville Pike
HFM-99, Suite 200 North
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Carol Walker
Regulatory Information Specialist
Division of Vaccines and
Related Products Applications
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (5) - 21 CFR 312
21 CFR 50.20, 50.25
FDA Forms 1571 & 1572

prevention of malaria is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After April 20, 1998, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[63 FR 13528, Mar. 20, 1998]

§ 310.548 Drug products containing colloidal silver ingredients or silver salts offered over-the-counter (OTC) for the treatment and/or prevention of disease.

(a) Colloidal silver ingredients and silver salts have been marketed in over-the-counter (OTC) drug products for the treatment and prevention of numerous disease conditions. There are serious and complicating aspects to many of the diseases these silver ingredients purport to treat or prevent. Further, there is a lack of adequate data to establish general recognition of the safety and effectiveness of colloidal silver ingredients or silver salts for OTC use in the treatment or prevention of any disease. These ingredients and salts include, but are not limited to, silver proteins, mild silver protein, strong silver protein, silver, silver ion, silver chloride, silver cyanide, silver iodide, silver oxide, and silver phosphate.

(b) Any OTC drug product containing colloidal silver ingredients or silver salts that is labeled, represented, or promoted for the treatment and/or prevention of any disease is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act) for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product containing colloidal silver or silver salts labeled, represented, or promoted for any OTC drug use is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs as set forth in part 312 of this chapter.

(d) After September 16, 1999, any such OTC drug product containing colloidal silver or silver salts initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[64 FR 44658, Aug. 17, 1999]

PART 312—INVESTIGATIONAL NEW DRUG APPLICATION

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312.160 Drugs for investigational use in laboratory research animals or in vitro tests.

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 371; 42 U.S.C. 262.

SOURCE: 52 FR 8831, Mar. 19, 1987, unless otherwise noted.

Subpart A—General Provisions

§ 312.1 Scope.

(a) This part contains procedures and requirements governing the use of investigational new drugs, including procedures and requirements for the submission to, and review by, the Food and Drug Administration of investigational new drug applications (IND's). An investigational new drug for which an IND is in effect in accordance with this part is exempt from the premarketing approval requirements that are otherwise applicable and may be shipped lawfully for the purpose of conducting clinical investigations of that drug.

(b) References in this part to regulations in the Code of Federal Regulations are to chapter I of title 21, unless otherwise noted.

§ 312.2 Applicability.

(a) *Applicability.* Except as provided in this section, this part applies to all clinical investigations of products that are subject to section 505 of the Federal Food, Drug, and Cosmetic Act or to the licensing provisions of the Public Health Service Act (58 Stat. 632, as amended (42 U.S.C. 201 *et seq.*)).

(b) *Exemptions.* (1) The clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the requirements of this part if all the following apply:

(i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) The investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

(v) The investigation is conducted in compliance with the requirements of § 312.7.

(2)(i) A clinical investigation involving an in vitro diagnostic biological product listed in paragraph (b)(2)(ii) of this section is exempt from the requirements of this part if (*a*) it is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure and (*b*) it is shipped in compliance with § 312.160.

(ii) In accordance with paragraph (b)(2)(i) of this section, the following products are exempt from the requirements of this part: (*a*) blood grouping serum; (*b*) reagent red blood cells; and (*c*) anti-human globulin.

(3) A drug intended solely for tests in vitro or in laboratory research animals is exempt from the requirements of this part if shipped in accordance with § 312.160.

(4) FDA will not accept an application for an investigation that is exempt under the provisions of paragraph (b)(1) of this section.

(5) A clinical investigation involving use of a placebo is exempt from the requirements of this part if the investigation does not otherwise require submission of an IND.

(6) A clinical investigation involving an exception from informed consent under § 50.24 of this chapter is not exempt from the requirements of this part.

(c) *Bioavailability studies.* The applicability of this part to in vivo bioavailability studies in humans is subject to the provisions of § 320.31.

(d) *Unlabeled indication.* This part does not apply to the use in the practice of medicine for an unlabeled indication of a new drug product approved under part 314 or of a licensed biological product.

(e) *Guidance.* FDA may, on its own initiative, issue guidance on the applicability of this part to particular investigational uses of drugs. On request, FDA will advise on the applicability of this part to a planned clinical investigation.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 64 FR 401, Jan. 5, 1999]

§ 312.3 Definitions and interpretations.

(a) The definitions and interpretations of terms contained in section 201 of the Act apply to those terms when used in this part:

(b) The following definitions of terms also apply to this part:

Act means the Federal Food, Drug, and Cosmetic Act (secs. 201-902, 52 Stat. 1040 *et seq.*, as amended (21 U.S.C. 301-392)).

Clinical investigation means any experiment in which a drug is administered or dispensed to, or used involving, one or more human subjects. For the purposes of this part, an experiment is any use of a drug except for the use of a marketed drug in the course of medical practice.

Contract research organization means a person that assumes, as an independent contractor with the sponsor, one or more of the obligations of a sponsor, e.g., design of a protocol, selection or monitoring of investigations, evaluation of reports, and preparation of materials to be submitted to the Food and Drug Administration.

FDA means the Food and Drug Administration.

IND means an investigational new drug application. For purposes of this part, "IND" is synonymous with "Notice of Claimed Investigational Exemption for a New Drug."

Investigational new drug means a new drug or biological drug that is used in a clinical investigation. The term also includes a biological product that is used in vitro for diagnostic purposes. The terms "investigational drug" and "investigational new drug" are deemed to be synonymous for purposes of this part.

Investigator means an individual who actually conducts a clinical investigation (i.e., under whose immediate direction the drug is administered or dispensed to a subject). In the event an investigation is conducted by a team of individuals, the investigator is the responsible leader of the team. "Subinvestigator" includes any other individual member of that team.

Marketing application means an application for a new drug submitted under section 505(b) of the act or a biologics license application for a biological product submitted under the Public Health Service Act.

Sponsor means a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization. The sponsor does not actually conduct the investigation unless the sponsor is a sponsor-investigator. A person other than an individual that uses one or more of its own employees to conduct an investigation that it has initiated is a sponsor, not a sponsor-investigator, and the employees are investigators.

Sponsor-Investigator means an individual who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. The term does not include any person other than an individual. The requirements applicable to a sponsor-investigator under this part include both those applicable to an investigator and a sponsor.

Subject means a human who participates in an investigation, either as a recipient of the investigational new drug or as a control. A subject may be a healthy human or a patient with a disease.

[52 FR 8831, Mar. 19, 1987, as amended at 64 FR 401, Jan. 5, 1999; 64 FR 56449, Oct. 20, 1999]

§ 312.6 Labeling of an investigational new drug.

(a) The immediate package of an investigational new drug intended for human use shall bear a label with the statement "Caution: New Drug—Limited by Federal (or United States) law to investigational use."

(b) The label or labeling of an investigational new drug shall not bear any statement that is false or misleading in any particular and shall not represent that the investigational new drug is safe or effective for the purposes for which it is being investigated.

§ 312.7 Promotion and charging for investigational drugs.

(a) *Promotion of an investigational new drug.* A sponsor or investigator, or any person acting on behalf of a sponsor or investigator, shall not represent in a promotional context that an investigational new drug is safe or effective for the purposes for which it is under investigation or otherwise promote the drug. This provision is not intended to restrict the full exchange of scientific information concerning the drug, including dissemination of scientific findings in scientific or lay media. Rather, its intent is to restrict promotional claims of safety or effectiveness of the drug for a use for which it is under investigation and to preclude commercialization of the drug before it is approved for commercial distribution.

(b) *Commercial distribution of an investigational new drug.* A sponsor or investigator shall not commercially distribute or test market an investigational new drug.

(c) *Prolonging an investigation.* A sponsor shall not unduly prolong an investigation after finding that the results of the investigation appear to establish sufficient data to support a marketing application.

(d) *Charging for and commercialization of investigational drugs*—(1) *Clinical trials under an IND.* Charging for an investigational drug in a clinical trial under an IND is not permitted without the prior written approval of FDA. In requesting such approval, the sponsor shall provide a full written explanation of why charging is necessary in order for the sponsor to undertake or continue the clinical trial, e.g., why distribution of the drug to test subjects should not be considered part of the normal cost of doing business.

(2) *Treatment protocol or treatment IND.* A sponsor or investigator may charge for an investigational drug for a treatment use under a treatment protocol or treatment IND provided: (i) There is adequate enrollment in the ongoing clinical investigations under the authorized IND; (ii) charging does not constitute commercial marketing of a new drug for which a marketing application has not been approved; (iii) the drug is not being commercially promoted or advertised; and (iv) the sponsor of the drug is actively pursuing marketing approval with due diligence. FDA must be notified in writing in advance of commencing any such charges, in an information amendment submitted under § 312.31. Authorization for charging goes into effect automatically 30 days after receipt by FDA of the information amendment, unless the sponsor is notified to the contrary.

(3) *Noncommercialization of investigational drug.* Under this section, the sponsor may not commercialize an investigational drug by charging a price larger than that necessary to recover costs of manufacture, research, development, and handling of the investigational drug.

(4) *Withdrawal of authorization.* Authorization to charge for an investigational drug under this section may be withdrawn by FDA if the agency finds that the conditions underlying the authorization are no longer satisfied.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19476, May 22, 1987; 67 FR 9585, Mar. 4, 2002]

§ 312.10 Waivers.

(a) A sponsor may request FDA to waive applicable requirement under this part. A waiver request may be submitted either in an IND or in an information amendment to an IND. In an emergency, a request may be made by telephone or other rapid communication means. A waiver request is required to contain at least one of the following:

(1) An explanation why the sponsor's compliance with the requirement is unnecessary or cannot be achieved;

(2) A description of an alternative submission or course of action that satisfies the purpose of the requirement; or

(3) Other information justifying a waiver.

(b) FDA may grant a waiver if it finds that the sponsor's noncompliance would not pose a significant and unreasonable risk to human subjects of the investigation and that one of the following is met:

(1) The sponsor's compliance with the requirement is unnecessary for the agency to evaluate the application, or compliance cannot be achieved;

(2) The sponsor's proposed alternative satisfies the requirement; or

(3) The applicant's submission otherwise justifies a waiver.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9585, Mar. 4, 2002]

Subpart B—Investigational New Drug Application (IND)

§ 312.20 Requirement for an IND.

(a) A sponsor shall submit an IND to FDA if the sponsor intends to conduct a clinical investigation with an investigational new drug that is subject to § 312.2(a).

(b) A sponsor shall not begin a clinical investigation subject to § 312.2(a) until the investigation is subject to an IND which is in effect in accordance with § 312.40.

(c) A sponsor shall submit a separate IND for any clinical investigation involving an exception from informed consent under § 50.24 of this chapter. Such a clinical investigation is not permitted to proceed without the prior written authorization from FDA. FDA shall provide a written determination 30 days after FDA receives the IND or earlier.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 62 FR 32479, June 16, 1997]

§ 312.21 Phases of an investigation.

An IND may be submitted for one or more phases of an investigation. The clinical investigation of a previously untested drug is generally divided into three phases. Although in general the phases are conducted sequentially, they may overlap. These three phases of an investigation are a follows:

(a) *Phase 1.* (1) Phase 1 includes the initial introduction of an investigational new drug into humans. Phase 1 studies are typically closely monitored and may be conducted in patients or normal volunteer subjects. These studies are designed to determine the metabolism and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. During Phase 1, sufficient

information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid, Phase 2 studies. The total number of subjects and patients included in Phase 1 studies varies with the drug, but is generally in the range of 20 to 80.

(2) Phase 1 studies also include studies of drug metabolism, structure-activity relationships, and mechanism of action in humans, as well as studies in which investigational drugs are used as research tools to explore biological phenomena or disease processes.

(b) *Phase 2.* Phase 2 includes the controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks associated with the drug. Phase 2 studies are typically well controlled, closely monitored, and conducted in a relatively small number of patients, usually involving no more than several hundred subjects.

(c) *Phase 3.* Phase 3 studies are expanded controlled and uncontrolled trials. They are performed after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug and to provide an adequate basis for physician labeling. Phase 3 studies usually include from several hundred to several thousand subjects.

§ 312.22 General principles of the IND submission.

(a) FDA's primary objectives in reviewing an IND are, in all phases of the investigation, to assure the safety and rights of subjects, and, in Phase 2 and 3, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety. Therefore, although FDA's review of Phase 1 submissions will focus on assessing the safety of Phase 1 investigations, FDA's review of Phases 2 and 3 submissions will also include an assessment of the scientific quality of the clinical investigations and the likelihood that the investigations will yield data capable of meeting statutory standards for marketing approval.

(b) The amount of information on a particular drug that must be submitted in an IND to assure the accomplishment of the objectives described in paragraph (a) of this section depends upon such factors as the novelty of the drug, the extent to which it has been studied previously, the known or suspected risks, and the developmental phase of the drug.

(c) The central focus of the initial IND submission should be on the general investigational plan and the protocols for specific human studies. Subsequent amendments to the IND that contain new or revised protocols should build logically on previous submissions and should be supported by additional information, including the results of animal toxicology studies or other human studies as appropriate. Annual reports to the IND should serve as the focus for reporting the status of studies being conducted under the IND and should update the general investigational plan for the coming year.

(d) The IND format set forth in § 312.23 should be followed routinely by sponsors in the interest of fostering an efficient review of applications. Sponsors are expected to exercise considerable discretion, however, regarding the content of information submitted in each section, depending upon the kind of drug being studied and the nature of the available information. Section 312.23 outlines the information needed for a commercially sponsored IND for a new molecular entity. A sponsor-investigator who uses, as a research tool, an investigational new drug that is already subject to a manufacturer's IND or marketing application should follow the same general format, but ordinarily may, if authorized by the manufacturer, refer to the manufacturer's IND or marketing application in providing the technical information supporting the proposed clinical investigation. A sponsor-investigator who uses an investigational drug not subject to a manufacturer's IND or marketing application is ordinarily required to submit all technical information supporting the IND, unless such information may be referenced from the scientific literature.

§ 312.23 IND content and format.

(a) A sponsor who intends to conduct a clinical investigation subject to this part shall submit an "Investigational New Drug Application" (IND) including, in the following order:

(1) *Cover sheet (Form FDA–1571).* A cover sheet for the application containing the following:

(i) The name, address, and telephone number of the sponsor, the date of the application, and the name of the investigational new drug.

(ii) Identification of the phase or phases of the clinical investigation to be conducted.

(iii) A commitment not to begin clinical investigations until an IND covering the investigations is in effect.

(iv) A commitment that an Institutional Review Board (IRB) that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of each of the studies in the proposed clinical investigation and that the investigator will report to the IRB proposed changes in the research activity in accordance with the requirements of part 56.

(v) A commitment to conduct the investigation in accordance with all other applicable regulatory requirements.

(vi) The name and title of the person responsible for monitoring the conduct and progress of the clinical investigations.

(vii) The name(s) and title(s) of the person(s) responsible under § 312.32 for review and evaluation of information relevant to the safety of the drug.

(viii) If a sponsor has transferred any obligations for the conduct of any clinical study to a contract research organization, a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred. If all obligations governing the conduct of the study have been transferred, a general statement of this transfer—in lieu of a listing of the specific obligations transferred—may be submitted.

(ix) The signature of the sponsor or the sponsor's authorized representative. If the person signing the application does not reside or have a place of business within the United States, the IND is required to contain the name and address of, and be countersigned by, an attorney, agent, or other authorized official who resides or maintains a place of business within the United States.

(2) *A table of contents.*

(3) *Introductory statement and general investigational plan.* (i) A brief introductory statement giving the name of the drug and all active ingredients, the drug's pharmacological class, the structural formula of the drug (if known), the formulation of the dosage form(s) to be used, the route of administration, and the broad objectives and planned duration of the proposed clinical investigation(s).

(ii) A brief summary of previous human experience with the drug, with reference to other IND's if pertinent, and to investigational or marketing experience in other countries that may be relevant to the safety of the proposed clinical investigation(s).

(iii) If the drug has been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness, identification of the country(ies) where the drug was withdrawn and the reasons for the withdrawal.

(iv) A brief description of the overall plan for investigating the drug product for the following year. The plan should include the following: (*a*) The rationale for the drug or the research study; (*b*) the indication(s) to be studied; (*c*) the general approach to be followed in evaluating the drug; (*d*) the kinds of clinical trials to be conducted in the first year following the submission (if plans are not developed for the entire year, the sponsor should so indicate); (*e*) the estimated number of patients to be given the drug in those studies; and (*f*) any risks of particular severity or seriousness anticipated on the basis of the toxicological data in animals or prior studies in humans with the drug or related drugs.

(4) [Reserved]

(5) *Investigator's brochure.* If required under § 312.55, a copy of the investigator's brochure, containing the following information:

(i) A brief description of the drug substance and the formulation, including the structural formula, if known.

(ii) A summary of the pharmacological and toxicological effects of the drug in animals and, to the extent known, in humans.

(iii) A summary of the pharmacokinetics and biological disposition of the drug in animals and, if known, in humans.

(iv) A summary of information relating to safety and effectiveness in humans obtained from prior clinical studies. (Reprints of published articles on such studies may be appended when useful.)

(v) A description of possible risks and side effects to be anticipated on the basis of prior experience with the drug under investigation or with related drugs, and of precautions or special monitoring to be done as part of the investigational use of the drug.

(6) *Protocols.* (i) A protocol for each planned study. (Protocols for studies not submitted initially in the IND should be submitted in accordance with § 312.30(a).) In general, protocols for Phase 1 studies may be less detailed and more flexible than protocols for Phase 2 and 3 studies. Phase 1 protocols should be directed primarily at providing an outline of the investigation—an estimate of the number of patients to be involved, a description of safety exclusions, and a description of the dosing plan including duration, dose, or method to be used in determining dose—and should specify in detail only those elements of the study that are critical to safety, such as necessary monitoring of vital signs and blood chemistries. Modifications of the experimental design of Phase 1 studies that do not affect critical safety assessments are required to be reported to FDA only in the annual report.

(ii) In Phases 2 and 3, detailed protocols describing all aspects of the study should be submitted. A protocol for a Phase 2 or 3 investigation should be designed in such a way that, if the sponsor anticipates that some deviation from the study design may become necessary as the investigation progresses, alternatives or contingencies to provide for such deviation are built into the protocols at the outset. For example, a protocol for a controlled short-term study might include a plan for an early crossover of nonresponders to an alternative therapy.

(iii) A protocol is required to contain the following, with the specific elements and detail of the protocol reflecting the above distinctions depending on the phase of study:

(*a*) A statement of the objectives and purpose of the study.

(*b*) The name and address and a statement of the qualifications (curriculum vitae or other statement of qualifications) of each investigator, and the name of each subinvestigator (e.g., research fellow, resident) working under the supervision of the investigator; the name and address of the research facilities to be used; and the name and address of each reviewing Institutional Review Board.

(*c*) The criteria for patient selection and for exclusion of patients and an estimate of the number of patients to be studied.

(*d*) A description of the design of the study, including the kind of control group to be used, if any, and a description of methods to be used to minimize bias on the part of subjects, investigators, and analysts.

(*e*) The method for determining the dose(s) to be administered, the planned maximum dosage, and the duration of individual patient exposure to the drug.

(*f*) A description of the observations and measurements to be made to fulfill the objectives of the study.

(*g*) A description of clinical procedures, laboratory tests, or other measures to be taken to monitor the effects of the drug in human subjects and to minimize risk.

(7) *Chemistry, manufacturing, and control information.* (i) As appropriate for the particular investigations covered by the IND, a section describing the composition, manufacture, and control of the drug substance and the drug product. Although in each phase of the investigation sufficient information is required to be submitted to assure the proper identification, quality, purity, and strength of the investigational drug, the amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available. FDA recognizes that modifications to the method of preparation of the new drug substance and dosage form and changes in the dosage form itself are likely as the investigation progresses. Therefore, the emphasis in an initial Phase 1 submission should generally be placed on the identification and control of the raw materials and the new drug substance. Final specifications for the drug substance and drug product are not expected until the end of the investigational process.

(ii) It should be emphasized that the amount of information to be submitted depends upon the scope of the proposed clinical investigation. For example, although stability data are required in all phases of the IND to demonstrate that the new drug substance and drug product are within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation, if very short-term tests are proposed, the supporting stability data can be correspondingly limited.

(iii) As drug development proceeds and as the scale or production is changed from the pilot-scale production appropriate for the limited initial clinical investigations to the larger-scale production needed for expanded clinical trials, the sponsor should submit information amendments to supplement the initial information submitted on the chemistry, manufacturing, and control processes with information appropriate to the expanded scope of the investigation.

(iv) Reflecting the distinctions described in this paragraph (a)(7), and based on the phase(s) to be studied, the submission is required to contain the following:

(*a*) *Drug substance.* A description of the drug substance, including its physical, chemical, or biological characteristics; the name and address of its manufacturer; the general method of preparation of the drug substance; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug substance; and information sufficient to support stability of the drug substance during the toxicological studies and the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy relevant requirements in this paragraph.

(*b*) *Drug product.* A list of all components, which may include reasonable alternatives for inactive compounds, used in the manufacture of the investigational drug product, including both those components intended to appear in the drug product and those which may not appear but which are used in the manufacturing process, and, where applicable, the quantitative composition of the investigational drug product, including any reasonable variations that may be expected during the investigational stage; the name and address of the drug product manufacturer; a brief general description of the manufacturing and packaging procedure as appropriate for the product; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug product; and information sufficient to assure the product's stability during the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy certain requirements in this paragraph.

(*c*) A brief general description of the composition, manufacture, and control of any placebo used in a controlled clinical trial.

(*d*) *Labeling.* A copy of all labels and labeling to be provided to each investigator.

(*e*) *Environmental analysis requirements.* A claim for categorical exclusion under § 25.30 or 25.31 or an environmental assessment under § 25.40.

(8) *Pharmacology and toxicology information.* Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or in vitro, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and

other tests required varies with the duration and nature of the proposed clinical investigations. Guidance documents are available from FDA that describe ways in which these requirements may be met. Such information is required to include the identification and qualifications of the individuals who evaluated the results of such studies and concluded that it is reasonably safe to begin the proposed investigations and a statement of where the investigations were conducted and where the records are available for inspection. As drug development proceeds, the sponsor is required to submit informational amendments, as appropriate, with additional information pertinent to safety.

(i) *Pharmacology and drug disposition.* A section describing the pharmacological effects and mechanism(s) of action of the drug in animals, and information on the absorption, distribution, metabolism, and excretion of the drug, if known.

(ii) *Toxicology.* (a) An integrated summary of the toxicological effects of the drug in animals and in vitro. Depending on the nature of the drug and the phase of the investigation, the description is to include the results of acute, subacute, and chronic toxicity tests; tests of the drug's effects on reproduction and the developing fetus; any special toxicity test related to the drug's particular mode of administration or conditions of use (e.g., inhalation, dermal, or ocular toxicology); and any in vitro studies intended to evaluate drug toxicity.

(b) For each toxicology study that is intended primarily to support the safety of the proposed clinical investigation, a full tabulation of data suitable for detailed review.

(iii) For each nonclinical laboratory study subject to the good laboratory practice regulations under part 58, a statement that the study was conducted in compliance with the good laboratory practice regulations in part 58, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance.

(9) *Previous human experience with the investigational drug.* A summary of previous human experience known to the applicant, if any, with the investigational drug. The information is required to include the following:

(i) If the investigational drug has been investigated or marketed previously, either in the United States or other countries, detailed information about such experience that is relevant to the safety of the proposed investigation or to the investigation's rationale. If the durg has been the subject of controlled trials, detailed information on such trials that is relevant to an assessment of the drug's effectiveness for the proposed investigational use(s) should also be provided. Any published material that is relevant to the safety of the proposed investigation or to an assessment of the drug's effectiveness for its proposed investigational use should be provided in full. Published material that is less directly relevant may be supplied by a bibliography.

(ii) If the drug is a combination of drugs previously investigated or marketed, the information required under paragraph (a)(9)(i) of this section should be provided for each active drug component. However, if any component in such combination is subject to an approved marketing application or is otherwise lawfully marketed in the United States, the sponsor is not required to submit published material concerning that active drug component unless such material relates directly to the proposed investigational use (including publications relevant to component-component interaction).

(iii) If the drug has been marketed outside the United States, a list of the countries in which the drug has been marketed and a list of the countries in which the drug has been withdrawn from marketing for reasons potentially related to safety or effectiveness.

(10) *Additional information.* In certain applications, as described below, information on special topics may be needed. Such information shall be submitted in this section as follows:

(i) *Drug dependence and abuse potential.* If the drug is a psychotropic substance or otherwise has abuse potential, a section describing relevant clinical studies and experience and studies in test animals.

(ii) *Radioactive drugs.* If the drug is a radioactive drug, sufficient data from animal or human studies to allow a reasonable calculation of radiation-absorbed dose to the whole body and critical organs upon administration to a human subject. Phase 1 studies of radioactive drugs must include studies which will obtain sufficient data for dosimetry calculations.

(iii) *Pediatric studies.* Plans for assessing pediatric safety and effectiveness.

(iv) *Other information.* A brief statement of any other information that would aid evaluation of the proposed clinical investigations with respect to their safety or their design and potential as controlled clinical trials to support marketing of the drug.

(11) *Relevant information.* If requested by FDA, any other relevant information needed for review of the application.

(b) *Information previously submitted.* The sponsor ordinarily is not required to resubmit information previously submitted, but may incorporate the information by reference. A reference to information submitted previously must identify the file by name, reference number, volume, and page number where the information can be found. A reference to information submitted to the agency by a person other than the sponsor is required to contain a written statement that authorizes the reference and that is signed by the person who submitted the information.

(c) *Material in a foreign language.* The sponsor shall submit an accurate and complete English translation of each part of the IND that is not in English. The sponsor shall also submit a copy of each original literature publication for which an English translation is submitted.

(d) *Number of copies.* The sponsor shall submit an original and two copies of all submissions to the IND file, including the original submission and all amendments and reports.

(e) *Numbering of IND submissions.* Each submission relating to an IND is required to be numbered serially using a single, three-digit serial number. The initial IND is required to be numbered 000; each subsequent submission (e.g., amendment, report, or correspondence) is required to be numbered chronologically in sequence.

(f) *Identification of exception from informed consent.* If the investigation involves an exception from informed consent under §50.24 of this chapter, the sponsor shall prominently identify on the cover sheet that the investigation is subject to the requirements in §50.24 of this chapter.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51529, Oct. 2, 1996; 62 FR 40599, July 29, 1997; 63 FR 66669, Dec. 2, 1998; 65 FR 56479, Sept. 19, 2000; 67 FR 9585, Mar. 4, 2002]

§ 312.30 Protocol amendments.

Once an IND is in effect, a sponsor shall amend it as needed to ensure that the clinical investigations are conducted according to protocols included in the application. This section sets forth the provisions under which new protocols may be submitted and changes in previously submitted protocols may be made. Whenever a sponsor intends to conduct a clinical investigation with an exception from informed consent for emergency research as set forth in §50.24 of this chapter, the sponsor shall submit a separate IND for such investigation.

(a) *New protocol.* Whenever a sponsor intends to conduct a study that is not covered by a protocol already contained in the IND, the sponsor shall submit to FDA a protocol amendment containing the protocol for the study. Such study may begin provided two conditions are met: (1) The sponsor has submitted the protocol to FDA for its review; and (2) the protocol has been approved by the Institutional Review Board (IRB) with responsibility for review and approval of the study in accordance with the requirements of part 56. The sponsor may comply with these two conditions in either order.

(b) *Changes in a protocol.* (1) A sponsor shall submit a protocol amendment describing any change in a Phase 1 protocol that significantly affects the safety of subjects or any change in a Phase 2 or 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of changes requiring an amendment under this paragraph include:

(i) Any increase in drug dosage or duration of exposure of individual subjects to the drug beyond that in the current protocol, or any significant increase in the number of subjects under study.

(ii) Any significant change in the design of a protocol (such as the addition or dropping of a control group).

(iii) The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor safety.

(2)(i) A protocol change under paragraph (b)(1) of this section may be made provided two conditions are met:

(*a*) The sponsor has submitted the change to FDA for its review; and

(*b*) The change has been approved by the IRB with responsibility for review and approval of the study. The sponsor may comply with these two conditions in either order.

(ii) Notwithstanding paragraph (b)(2)(i) of this section, a protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately provided FDA is subsequently notified by protocol amendment and the reviewing IRB is notified in accordance with § 56.104(c).

(c) *New investigator.* A sponsor shall submit a protocol amendment when a new investigator is added to carry out a previously submitted protocol, except that a protocol amendment is not required when a licensed practitioner is added in the case of a treatment protocol under § 312.34. Once the investigator is added to the study, the investigational drug may be shipped to the investigator and the investigator may begin participating in the study. The sponsor shall notify FDA of the new investigator within 30 days of the investigator being added.

(d) *Content and format.* A protocol amendment is required to be prominently identified as such (i.e., "Protocol Amendment: New Protocol", "Protocol Amendment: Change in Protocol", or "Protocol Amendment: New Investigator"), and to contain the following:

(1)(i) In the case of a new protocol, a copy of the new protocol and a brief description of the most clinically significant differences between it and previous protocols.

(ii) In the case of a change in protocol, a brief description of the change and reference (date and number) to the submission that contained the protocol.

(iii) In the case of a new investigator, the investigator's name, the qualifications to conduct the investigation, reference to the previously submitted protocol, and all additional information about the investigator's study as is required under § 312.23(a)(6)(iii)(*b*).

(2) Reference, if necessary, to specific technical information in the IND or in a concurrently submitted information amendment to the IND that the sponsor relies on to support any clinically significant change in the new or amended protocol. If the reference is made to supporting information already in the IND, the sponsor shall identify by name, reference number, volume, and page number the location of the information.

(3) If the sponsor desires FDA to comment on the submission, a request for such comment and the specific questions FDA's response should address.

(e) *When submitted.* A sponsor shall submit a protocol amendment for a new protocol or a change in protocol before its implementation. Protocol amendments to add a new investigator or to provide additional information about investigators may be grouped and submitted at 30-day intervals. When several submissions of new protocols or protocol changes are anticipated during a short period, the sponsor is encouraged, to the extent feasible, to include these all in a single submission.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51530, Oct. 2, 1996; 67 FR 9585, Mar. 4, 2002]

§ 312.31 Information amendments.

(a) *Requirement for information amendment.* A sponsor shall report in an information amendment essential information on the IND that is not within the scope of a protocol amendment, IND safety reports, or annual report. Examples of information requiring an information amendment include:

(1) New toxicology, chemistry, or other technical information; or

(2) A report regarding the discontinuance of a clinical investigation.

(b) *Content and format of an information amendment.* An information amendment is required to bear prominent identification of its contents (e.g., "Information Amendment: Chemistry, Manufacturing, and Control", "Information Amendment: Pharmacology-Toxicology", "Information Amendment: Clinical"), and to contain the following:

(1) A statement of the nature and purpose of the amendment.

(2) An organized submission of the data in a format appropriate for scientific review.

(3) If the sponsor desires FDA to comment on an information amendment, a request for such comment.

(c) *When submitted.* Information amendments to the IND should be submitted as necessary but, to the extent feasible, not more than every 30 days.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 67 FR 9585, Mar. 4, 2002]

§ 312.32 IND safety reports.

(a) *Definitions.* The following definitions of terms apply to this section:

Associated with the use of the drug. There is a reasonable possibility that the experience may have been caused by the drug.

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience. Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse drug experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) *Review of safety information.* The sponsor shall promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from any source, foreign or domestic, including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities that have not already been previously reported to the agency by the sponsor.

(c) *IND safety reports.* (1) *Written reports*—(i) The sponsor shall notify FDA and all participating investigators in a written IND safety report of:

(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or

(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., "IND Safety Report." Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

(ii) In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previouos, similar reports.

(2) *Telephone and facsimile transmission safety reports.* The sponsor shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than 7 calendar days after the sponsor's initial receipt of the information. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

(3) *Reporting format or frequency.* FDA may request a sponsor to submit IND safety reports in a format or at a frequency different than that required under this paragraph. The sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the new drug review division in the Center for Drug Evaluation and Research or the director of the products review division in the Center for Biologics Evaluation and Research which is responsible for review of the IND.

(4) A sponsor of a clinical study of a marketed drug is not required to make a safety report for any adverse experience associated with use of the drug that is not from the clinical study itself.

(d) *Followup.* (1) The sponsor shall promptly investigate all safety information received by it.

(2) Followup information to a safety report shall be submitted as soon as the relevant information is available.

(3) If the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable under paragraph (c) of this section is so reportable, the sponsor shall report such experience in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

(4) Results of a sponsor's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report.

(e) *Disclaimer.* A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse experience. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse experience.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 62 FR 52250, Oct. 7, 1997; 67 FR 9585, Mar. 4, 2002]

§ 312.33 Annual reports.

A sponsor shall within 60 days of the anniversary date that the IND went into effect, submit a brief report of the progress of the investigation that includes:

(a) *Individual study information.* A brief summary of the status of each study in progress and each study completed during the previous year. The summary is required to include the following information for each study:

(1) The title of the study (with any appropriate study identifiers such as protocol number), its purpose, a brief statement identifying the patient population, and a statement as to whether the study is completed.

(2) The total number of subjects initially planned for inclusion in the study; the number entered into the study to date, tabulated by age group, gender, and race; the number whose participation in the study was completed as planned; and the number who dropped out of the study for any reason.

(3) If the study has been completed, or if interim results are known, a brief description of any available study results.

(b) *Summary information.* Information obtained during the previous year's clinical and nonclinical investigations, including:

(1) A narrative or tabular summary showing the most frequent and most serious adverse experiences by body system.

(2) A summary of all IND safety reports submitted during the past year.

(3) A list of subjects who died during participation in the investigation, with the cause of death for each subject.

(4) A list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related.

(5) A brief description of what, if anything, was obtained that is pertinent to an understanding of the drug's actions, including, for example, information about dose response, information from controlled trails, and information about bioavailability.

(6) A list of the preclinical studies (including animal studies) completed or in progress during the past year and a summary of the major preclinical findings.

(7) A summary of any significant manufacturing or microbiological changes made during the past year.

(c) A description of the general investigational plan for the coming year to replace that submitted 1 year earlier. The general investigational plan shall contain the information required under § 312.23(a)(3)(iv).

(d) If the investigator brochure has been revised, a description of the revision and a copy of the new brochure.

(e) A description of any significant Phase 1 protocol modifications made during the previous year and not previously reported to the IND in a protocol amendment.

(f) A brief summary of significant foreign marketing developments with the drug during the past year, such as approval of marketing in any country or withdrawal or suspension from marketing in any country.

(g) If desired by the sponsor, a log of any outstanding business with respect to the IND for which the sponsor requests or expects a reply, comment, or meeting.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 6882, Feb. 11, 1998; 67 FR 9585, Mar. 4, 2002]

§ 312.34 Treatment use of an investigational new drug.

(a) *General.* A drug that is not approved for marketing may be under clinical investigation for a serious or immediately life-threatening disease condition in patients for whom no comparable or satisfactory alternative drug or other therapy is available. During the clinical investigation of the drug, it may be appropriate to use the drug in the treatment of patients not in the clinical trials, in accordance with a treatment protocol or treatment IND. The purpose of this section is to facilitate the availability of promising new drugs to desperately ill

patients as early in the drug development process as possible, before general marketing begins, and to obtain additional data on the drug's safety and effectiveness. In the case of a serious disease, a drug ordinarily may be made available for treatment use under this section during Phase 3 investigations or after all clinical trials have been completed; however, in appropriate circumstances, a drug may be made available for treatment use during Phase 2. In the case of an immediately life-threatening disease, a drug may be made available for treatment use under this section earlier than Phase 3, but ordinarily not earlier than Phase 2. For purposes of this section, the "treatment use" of a drug includes the use of a drug for diagnostic purposes. If a protocol for an investigational drug meets the criteria of this section, the protocol is to be submitted as a treatment protocol under the provisions of this section.

(b) *Criteria.* (1) FDA shall permit an investigational drug to be used for a treatment use under a treatment protocol or treatment IND if:

(i) The drug is intended to treat a serious or immediately life-threatening disease;

(ii) There is no comparable or satisfactory alternative drug or other therapy available to treat that stage of the disease in the intended patient population;

(iii) The drug is under investigation in a controlled clinical trial under an IND in effect for the trial, or all clinical trials have been completed; and

(iv) The sponsor of the controlled clinical trial is actively pursuing marketing approval of the investigational drug with due diligence.

(2) *Serious disease.* For a drug intended to treat a serious disease, the Commissioner may deny a request for treatment use under a treatment protocol or treatment IND if there is insufficient evidence of safety and effectiveness to support such use.

(3) *Immediately life-threatening disease.* (i) For a drug intended to treat an immediately life-threatening disease, the Commissioner may deny a request for treatment use of an investigational drug under a treatment protocol or treatment IND if the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(A) May be effective for its intended use in its intended patient population; or

(B) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(ii) For the purpose of this section, an "immediately life-threatening" disease means a stage of a disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment.

(c) *Safeguards.* Treatment use of an investigational drug is conditioned on the sponsor and investigators complying with the safeguards of the IND process, including the regulations governing informed consent (21 CFR part 50) and institutional review boards (21 CFR part 56) and the applicable provisions of part 312, including distribution of the drug through qualified experts, maintenance of adequate manufacturing facilities, and submission of IND safety reports.

(d) *Clinical hold.* FDA may place on clinical hold a proposed or ongoing treatment protocol or treatment IND in accordance with § 312.42.

[52 FR 19476, May 22, 1987, as amended at 57 FR 13248, Apr. 15, 1992]

§ 312.35 Submissions for treatment use.

(a) *Treatment protocol submitted by IND sponsor.* Any sponsor of a clinical investigation of a drug who intends to sponsor a treatment use for the drug shall submit to FDA a treatment protocol under § 312.34 if the sponsor believes the criteria of § 312.34 are satisfied. If a protocol is not submitted under § 312.34, but FDA believes that the protocol should have been submitted under this section, FDA may deem the protocol to be submitted under § 312.34. A treatment use under a treatment protocol may begin 30 days after FDA receives the protocol or on earlier notification by FDA that the treatment use described in the protocol may begin.

(1) A treatment protocol is required to contain the following:

(i) The intended use of the drug.

(ii) An explanation of the rationale for use of the drug, including, as appropriate, either a list of what available regimens ordinarily should be tried before using the investigational drug or an explanation of why the use of the investigational drug is preferable to the use of available marketed treatments.

(iii) A brief description of the criteria for patient selection.

(iv) The method of administration of the drug and the dosages.

(v) A description of clinical procedures, laboratory tests, or other measures to monitor the effects of the drug and to minimize risk.

(2) A treatment protocol is to be supported by the following:

(i) Informational brochure for supplying to each treating physician.

(ii) The technical information that is relevant to safety and effectiveness of the drug for the intended treatment purpose. Information contained in the sponsor's IND may be incorporated by reference.

(iii) A commitment by the sponsor to assure compliance of all participating investigators with the informed consent requirements of 21 CFR part 50.

(3) A licensed practioner who receives an investigational drug for treatment use under a treatment protocol is an "investigator" under the protocol and is responsible for meeting all applicable investigator responsibilities under this part and 21 CFR parts 50 and 56.

(b) *Treatment IND submitted by licensed practitioner.* (1) If a licensed medical practitioner wants to obtain an investigational drug subject to a controlled clinical trial for a treatment use, the practitioner should first attempt to obtain the drug from the sponsor of the controlled trial under a treatment protocol. If the sponsor of the controlled clinical investigation of the drug will not establish a treatment protocol for the drug under paragraph (a) of this section, the licensed medical practitioner may seek to obtain the drug from the sponsor and submit a treatment IND to FDA requesting authorization to use the investigational drug for treatment use. A treatment use under a treatment IND may begin 30 days after FDA receives the IND or on earlier notification by FDA that the treatment use under the IND may begin. A treatment IND is required to contain the following:

(i) A cover sheet (Form FDA 1571) meeting § 312.23(g)(1).

(ii) Information (when not provided by the sponsor) on the drug's chemistry, manufacturing, and controls, and prior clinical and nonclinical experience with the drug submitted in accordance with § 312.23. A sponsor of a clinical investigation subject to an IND who supplies an investigational drug to a licensed medical practitioner for purposes of a separate treatment clinical investigation shall be deemed to authorize the incorporation-by-reference of the technical information contained in the sponsor's IND into the medical practitioner's treatment IND.

(iii) A statement of the steps taken by the practitioner to obtain the drug under a treatment protocol from the drug sponsor.

(iv) A treatment protocol containing the same information listed in paragraph (a)(1) of this section.

(v) A statement of the practitioner's qualifications to use the investigational drug for the intended treatment use.

(vi) The practitioner's statement of familiarity with information on the drug's safety and effectiveness derived from previous clinical and nonclinical experience with the drug.

(vii) Agreement to report to FDA safety information in accordance with § 312.32.

(2) A licensed practitioner who submits a treatment IND under this section is the sponsor-investigator for such IND and is responsible for meeting all applicable sponsor and investigator responsibilities under this part and 21 CFR parts 50 and 56.

[52 FR 19477, May 22, 1987, as amended at 57 FR 13249, Apr. 15, 1992; 67 FR 9585, Mar. 4, 2002]

§ 312.36 Emergency use of an investigational new drug.

Need for an investigational drug may arise in an emergency situation that does not allow time for submission of an IND in accordance with § 312.23 or

§ 312.34. In such a case, FDA may authorize shipment of the drug for a specified use in advance of submission of an IND. A request for such authorization may be transmitted to FDA by telephone or other rapid communication means. For investigational biological drugs, the request should be directed to the Division of Biological Investigational New Drugs (HFB–230), Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892, 301–443–4864. For all other investigational drugs, the request for authorization should be directed to the Document Management and Reporting Branch (HFD–53), Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857, 301–443–4320. After normal working hours, eastern standard time, the request should be directed to the FDA Division of Emergency and Epidemiological Operations, 202–857–8400. Except in extraordinary circumstances, such authorization will be conditioned on the sponsor making an appropriate IND submission as soon as practicable after receiving the authorization.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 67 FR 9585, Mar. 4, 2002]

§ 312.38 Withdrawal of an IND.

(a) At any time a sponsor may withdraw an effective IND without prejudice.

(b) If an IND is withdrawn, FDA shall be so notified, all clinical investigations conducted under the IND shall be ended, all current investigators notified, and all stocks of the drug returned to the sponsor or otherwise disposed of at the request of the sponsor in accordance with § 312.59.

(c) If an IND is withdrawn because of a safety reason, the sponsor shall promptly so inform FDA, all participating investigators, and all reviewing Institutional Review Boards, together with the reasons for such withdrawal.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

Subpart C—Administrative Actions

§ 312.40 General requirements for use of an investigational new drug in a clinical investigation.

(a) An investigational new drug may be used in a clinical investigation if the following conditions are met:

(1) The sponsor of the investigation submits an IND for the drug to FDA; the IND is in effect under paragraph (b) of this section; and the sponsor complies with all applicable requirements in this part and parts 50 and 56 with respect to the conduct of the clinical investigations; and

(2) Each participating investigator conducts his or her investigation in compliance with the requirements of this part and parts 50 and 56.

(b) An IND goes into effect:

(1) Thirty days after FDA receives the IND, unless FDA notifies the sponsor that the investigations described in the IND are subject to a clinical hold under § 312.42; or

(2) On earlier notification by FDA that the clinical investigations in the IND may begin. FDA will notify the sponsor in writing of the date it receives the IND.

(c) A sponsor may ship an investigational new drug to investigators named in the IND:

(1) Thirty days after FDA receives the IND; or

(2) On earlier FDA authorization to ship the drug.

(d) An investigator may not administer an investigational new drug to human subjects until the IND goes into effect under paragraph (b) of this section.

§ 312.41 Comment and advice on an IND.

(a) FDA may at any time during the course of the investigation communicate with the sponsor orally or in writing about deficiencies in the IND or about FDA's need for more data or information.

(b) On the sponsor's request, FDA will provide advice on specific matters relating to an IND. Examples of such advice may include advice on the adequacy of technical data to support an

investigational plan, on the design of a clinical trial, and on whether proposed investigations are likely to produce the data and information that is needed to meet requirements for a marketing application.

(c) Unless the communication is accompanied by a clinical hold order under § 312.42, FDA communications with a sponsor under this section are solely advisory and do not require any modification in the planned or ongoing clinical investigations or response to the agency.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

§ 312.42 Clinical holds and requests for modification.

(a) *General.* A clinical hold is an order issued by FDA to the sponsor to delay a proposed clinical investigation or to suspend an ongoing investigation. The clinical hold order may apply to one or more of the investigations covered by an IND. When a proposed study is placed on clinical hold, subjects may not be given the investigational drug. When an ongoing study is placed on clinical hold, no new subjects may be recruited to the study and placed on the investigational drug; patients already in the study should be taken off therapy involving the investigational drug unless specifically permitted by FDA in the interest of patient safety.

(b) *Grounds for imposition of clinical hold.*—(1) *Clinical hold of a Phase 1 study under an IND.* FDA may place a proposed or ongoing Phase 1 investigation on clinical hold if it finds that:

(i) Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury;

(ii) The clinical investigators named in the IND are not qualified by reason of their scientific training and experience to conduct the investigation described in the IND;

(iii) The investigator brochure is misleading, erroneous, or materially incomplete; or

(iv) The IND does not contain sufficient information required under § 312.23 to assess the risks to subjects of the proposed studies.

(v) The IND is for the study of an investigational drug intended to treat a life-threatening disease or condition that affects both genders, and men or women with reproductive potential who have the disease or condition being studied are excluded from eligibility because of a risk or potential risk from use of the investigational drug of reproductive toxicity (i.e., affecting reproductive organs) or developmental toxicity (i.e., affecting potential offspring). The phrase "women with reproductive potential" does not include pregnant women. For purposes of this paragraph, "life-threatening illnesses or diseases" are defined as "diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted." The clinical hold would not apply under this paragraph to clinical studies conducted:

(A) Under special circumstances, such as studies pertinent only to one gender (e.g., studies evaluating the excretion of a drug in semen or the effects on menstrual function);

(B) Only in men or women, as long as a study that does not exclude members of the other gender with reproductive potential is being conducted concurrently, has been conducted, or will take place within a reasonable time agreed upon by the agency; or

(C) Only in subjects who do not suffer from the disease or condition for which the drug is being studied.

(2) *Clinical hold of a Phase 2 or 3 study under an IND.* FDA may place a proposed or ongoing Phase 2 or 3 investigation on clinical hold if it finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (b)(1)(v) of this section apply; or

(ii) The plan or protocol for the investigation is clearly deficient in design to meet its stated objectives.

(3) Clinical hold of a treatment IND or treatment protocol.

(i) *Proposed use.* FDA may place a proposed treatment IND or treatment protocol on clinical hold if it is determined that:

(A) The pertinent criteria in § 312.34(b) for permitting the treatment use to begin are not satisfied; or

(B) The treatment protocol or treatment IND does not contain the information required under § 312.35 (a) or (b)

to make the specified determination under §312.34(b).

(ii) *Ongoing use.* FDA may place an ongoing treatment protocol or treatment IND on clinical hold if it is determined that:

(A) There becomes available a comparable or satisfactory alternative drug or other therapy to treat that stage of the disease in the intended patient population for which the investigational drug is being used;

(B) The investigational drug is not under investigation in a controlled clinical trial under an IND in effect for the trial and not all controlled clinical trials necessary to support a marketing application have been completed, or a clinical study under the IND has been placed on clinical hold:

(C) The sponsor of the controlled clinical trial is not pursuing marketing approval with due diligence;

(D) If the treatment IND or treatment protocol is intended for a serious disease, there is insufficient evidence of safety and effectiveness to support such use; or

(E) If the treatment protocol or treatment IND was based on an immediately life-threatening disease, the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(*1*) May be effective for its intended use in its intended population; or

(2) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(iii) FDA may place a proposed or ongoing treatment IND or treatment protocol on clinical hold if it finds that any of the conditions in paragraph (b)(4)(i) through (b)(4)(viii) of this section apply.

(4) *Clinical hold of any study that is not designed to be adequate and well-controlled.* FDA may place a proposed or ongoing investigation that is not designed to be adequate and well-controlled on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1) or (b)(2) of this section apply; or

(ii) There is reasonable evidence the investigation that is not designed to be adequate and well-controlled is impeding enrollment in, or otherwise interfering with the conduct or completion of, a study that is designed to be an adequate and well-controlled investigation of the same or another investigational drug; or

(iii) Insufficient quantities of the investigational drug exist to adequately conduct both the investigation that is not designed to be adequate and well-controlled and the investigations that are designed to be adequate and well-controlled; or

(iv) The drug has been studied in one or more adequate and well-controlled investigations that strongly suggest lack of effectiveness; or

(v) Another drug under investigation or approved for the same indication and available to the same patient population has demonstrated a better potential benefit/risk balance; or

(vi) The drug has received marketing approval for the same indication in the same patient population; or

(vii) The sponsor of the study that is designed to be an adequate and well-controlled investigation is not actively pursuing marketing approval of the investigational drug with due diligence; or

(viii) The Commissioner determines that it would not be in the public interest for the study to be conducted or continued. FDA ordinarily intends that clinical holds under paragraphs (b)(4)(ii), (b)(4)(iii) and (b)(4)(v) of this section would only apply to additional enrollment in nonconcurrently controlled trials rather than eliminating continued access to individuals already receiving the investigational drug.

(5) *Clinical hold of any investigation involving an exception from informed consent under §50.24 of this chapter.* FDA may place a proposed or ongoing investigation involving an exception from informed consent under §50.24 of this chapter on clinical hold if it is determined that:

(i) Any of the conditions in paragraphs (b)(1) or (b)(2) of this section apply; or

(ii) The pertinent criteria in §50.24 of this chapter for such an investigation to begin or continue are not submitted or not satisfied.

(6) Clinical hold of any investigation involving an exception from informed consent under §50.23(d) of this chapter. FDA may place a proposed or ongoing investigation involving an exception from informed consent under §50.23(d) of this chapter on clinical hold if it is determined that:

(i) Any of the conditions in paragraphs (b)(1) or (b)(2) of this section apply; or

(ii) A determination by the President to waive the prior consent requirement for the administration of an investigational new drug has not been made.

(c) *Discussion of deficiency.* Whenever FDA concludes that a deficiency exists in a clinical investigation that may be grounds for the imposition of clinical hold FDA will, unless patients are exposed to immediate and serious risk, attempt to discuss and satisfactorily resolve the matter with the sponsor before issuing the clinical hold order.

(d) *Imposition of clinical hold.* The clinical hold order may be made by telephone or other means of rapid communication or in writing. The clinical hold order will identify the studies under the IND to which the hold applies, and will briefly explain the basis for the action. The clinical hold order will be made by or on behalf of the Division Director with responsibility for review of the IND. As soon as possible, and no more than 30 days after imposition of the clinical hold, the Division Director will provide the sponsor a written explanation of the basis for the hold.

(e) *Resumption of clinical investigations.* An investigation may only resume after FDA (usually the Division Director, or the Director's designee, with responsibility for review of the IND) has notified the sponsor that the investigation may proceed. Resumption of the affected investigation(s) will be authorized when the sponsor corrects the deficiency(ies) previously cited or otherwise satisfies the agency that the investigation(s) can proceed. FDA may notify a sponsor of its determination regarding the clinical hold by telephone or other means of rapid communication. If a sponsor of an IND that has been placed on clinical hold requests in writing that the clinical hold be removed and submits a complete response to the issue(s) identified in the clinical hold order, FDA shall respond in writing to the sponsor within 30-calendar days of receipt of the request and the complete response. FDA's response will either remove or maintain the clinical hold, and will state the reasons for such determination. Notwithstanding the 30-calendar day response time, a sponsor may not proceed with a clinical trial on which a clinical hold has been imposed until the sponsor has been notified by FDA that the hold has been lifted.

(f) *Appeal.* If the sponsor disagrees with the reasons cited for the clinical hold, the sponsor may request reconsideration of the decision in accordance with §312.48.

(g) *Conversion of IND on clinical hold to inactive status.* If all investigations covered by an IND remain on clinical hold for 1 year or more, the IND may be placed on inactive status by FDA under §312.45.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19477, May 22, 1987; 57 FR 13249, Apr. 15, 1992; 61 FR 51530, Oct. 2, 1996; 63 FR 68678, Dec. 14, 1998; 64 FR 54189, Oct. 5, 1999; 65 FR 34971, June 1, 2000]

§ 312.44 Termination.

(a) *General.* This section describes the procedures under which FDA may terminate an IND. If an IND is terminated, the sponsor shall end all clinical investigations conducted under the IND and recall or otherwise provide for the disposition of all unused supplies of the drug. A termination action may be based on deficiencies in the IND or in the conduct of an investigation under an IND. Except as provided in paragraph (d) of this section, a termination shall be preceded by a proposal to terminate by FDA and an opportunity for the sponsor to respond. FDA will, in general, only initiate an action under this section after first attempting to resolve differences informally or, when appropriate, through the clinical hold procedures described in §312.42.

(b) *Grounds for termination*—(1) *Phase 1.* FDA may propose to terminate an IND during Phase 1 if it finds that:

(i) Human subjects would be exposed to an unreasonable and significant risk of illness or unjury.

(ii) The IND does not contain sufficient information required under §312.23 to assess the safety to subjects of the clinical investigations.

(iii) The methods, facilities, and controls used for the manufacturing, processing, and packing of the investigational drug are inadequate to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for subject safety.

(iv) The clinical investigations are being conducted in a manner substantially different than that described in the protocols submitted in the IND.

(v) The drug is being promoted or distributed for commercial purposes not justified by the requirements of the investigation or permitted by § 312.7.

(vi) The IND, or any amendment or report to the IND, contains an untrue statement of a material fact or omits material information required by this part.

(vii) The sponsor fails promptly to investigate and inform the Food and Drug Administration and all investigators of serious and unexpected adverse experiences in accordance with § 312.32 or fails to make any other report required under this part.

(viii) The sponsor fails to submit an accurate annual report of the investigations in accordance with § 312.33.

(ix) The sponsor fails to comply with any other applicable requirement of this part, part 50, or part 56.

(x) The IND has remained on inactive status for 5 years or more.

(xi) The sponsor fails to delay a proposed investigation under the IND or to suspend an ongoing investigation that has been placed on clinical hold under § 312.42(b)(4).

(2) *Phase 2 or 3.* FDA may propose to terminate an IND during Phase 2 or Phase 3 if FDA finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (b)(1)(xi) of this section apply; or

(ii) The investigational plan or protocol(s) is not reasonable as a bona fide scientific plan to determine whether or not the drug is safe and effective for use; or

(iii) There is convincing evidence that the drug is not effective for the purpose for which it is being investigated.

(3) FDA may propose to terminate a treatment IND if it finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (x) of this section apply; or

(ii) Any of the conditions in § 312.42(b)(3) apply.

(c) *Opportunity for sponsor response.* (1) If FDA proposes to terminate an IND, FDA will notify the sponsor in writing, and invite correction or explanation within a period of 30 days.

(2) On such notification, the sponsor may provide a written explanation or correction or may request a conference with FDA to provide the requested explanation or correction. If the sponsor does not respond to the notification within the allocated time, the IND shall be terminated.

(3) If the sponsor responds but FDA does not accept the explanation or correction submitted, FDA shall inform the sponsor in writing of the reason for the nonacceptance and provide the sponsor with an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be terminated. The sponsor's request for a regulatory hearing must be made within 10 days of the sponsor's receipt of FDA's notification of nonacceptance.

(d) *Immediate termination of IND.* Notwithstanding paragraphs (a) through (c) of this section, if at any time FDA concludes that continuation of the investigation presents an immediate and substantial danger to the health of individuals, the agency shall immediately, by written notice to the sponsor from the Director of the Center for Drug Evaluation and Research or the Director of the Center for Biologics Evaluation and Research, terminate the IND. An IND so terminated is subject to reinstatement by the Director on the basis of additional submissions that eliminate such danger. If an IND is terminated under this paragraph, the agency will afford the sponsor an opportunity for a regulatory hearing under part 16 on the question of whether the IND should be reinstated.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 57 FR 13249, Apr. 15, 1992; 67 FR 9586, Mar. 4, 2002]

§ 312.45 Inactive status.

(a) If no subjects are entered into clinical studies for a period of 2 years or more under an IND, or if all investigations under an IND remain on clinical hold for 1 year or more, the IND may be placed by FDA on inactive status. This action may be taken by FDA either on request of the sponsor or on FDA's own initiative. If FDA seeks to act on its own initiative under this section, it shall first notify the sponsor in writing of the proposed inactive status. Upon receipt of such notification, the sponsor shall have 30 days to respond as to why the IND should continue to remain active.

(b) If an IND is placed on inactive status, all investigators shall be so notified and all stocks of the drug shall be returned or otherwise disposed of in accordance with § 312.59.

(c) A sponsor is not required to submit annual reports to an IND on inactive status. An inactive IND is, however, still in effect for purposes of the public disclosure of data and information under § 312.130.

(d) A sponsor who intends to resume clinical investigation under an IND placed on inactive status shall submit a protocol amendment under § 312.30 containing the proposed general investigational plan for the coming year and appropriate protocols. If the protocol amendment relies on information previously submitted, the plan shall reference such information. Additional information supporting the proposed investigation, if any, shall be submitted in an information amendment. Notwithstanding the provisions of § 312.30, clinical investigations under an IND on inactive status may only resume (1) 30 days after FDA receives the protocol amendment, unless FDA notifies the sponsor that the investigations described in the amendment are subject to a clinical hold under § 312.42, or (2) on earlier notification by FDA that the clinical investigations described in the protocol amendment may begin.

(e) An IND that remains on inactive status for 5 years or more may be terminated under § 312.44.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

§ 312.47 Meetings.

(a) *General.* Meetings between a sponsor and the agency are frequently useful in resolving questions and issues raised during the course of a clinical investigation. FDA encourages such meetings to the extent that they aid in the evaluation of the drug and in the solution of scientific problems concerning the drug, to the extent that FDA's resources permit. The general principle underlying the conduct of such meetings is that there should be free, full, and open communication about any scientific or medical question that may arise during the clinical investigation. These meetings shall be conducted and documented in accordance with part 10.

(b) *"End-of-Phase 2" meetings and meetings held before submission of a marketing application.* At specific times during the drug investigation process, meetings between FDA and a sponsor can be especially helpful in minimizing wasteful expenditures of time and money and thus in speeding the drug development and evaluation process. In particular, FDA has found that meetings at the end of Phase 2 of an investigation (end-of-Phase 2 meetings) are of considerable assistance in planning later studies and that meetings held near completion of Phase 3 and before submission of a marketing application ("pre-NDA" meetings) are helpful in developing methods of presentation and submission of data in the marketing application that facilitate review and allow timely FDA response.

(1) *End-of-Phase 2 meetings*—(i) *Purpose.* The purpose of an end-of-phase 2 meeting is to determine the safety of proceeding to Phase 3, to evaluate the Phase 3 plan and protocols and the adequacy of current studies and plans to assess pediatric safety and effectiveness, and to identify any additional information necessary to support a marketing application for the uses under investigation.

(ii) *Eligibility for meeting.* While the end-of-Phase 2 meeting is designed primarily for IND's involving new molecular entities or major new uses of marketed drugs, a sponsor of any IND may request and obtain an end-of-Phase 2 meeting.

(iii) *Timing.* To be most useful to the sponsor, end-of-Phase 2 meetings should be held before major commitments of effort and resources to specific Phase 3 tests are made. The scheduling of an end-of-Phase 2 meeting is not, however, intended to delay the transition of an investigation from Phase 2 to Phase 3.

(iv) *Advance information.* At least 1 month in advance of an end-of-Phase 2 meeting, the sponsor should submit background information on the sponsor's plan for Phase 3, including summaries of the Phase 1 and 2 investigations, the specific protocols for Phase 3 clinical studies, plans for any additional nonclinical studies, plans for pediatric studies, including a time line for protocol finalization, enrollment, completion, and data analysis, or information to support any planned request for waiver or deferral of pediatric studies, and, if available, tentative labeling for the drug. The recommended contents of such a submission are described more fully in FDA Staff Manual Guide 4850.7 that is publicly available under FDA's public information regulations in part 20.

(v) *Conduct of meeting.* Arrangements for an end-of-Phase 2 meeting are to be made with the division in FDA's Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research which is responsible for review of the IND. The meeting will be scheduled by FDA at a time convenient to both FDA and the sponsor. Both the sponsor and FDA may bring consultants to the meeting. The meeting should be directed primarily at establishing agreement between FDA and the sponsor of the overall plan for Phase 3 and the objectives and design of particular studies. The adequacy of the technical information to support Phase 3 studies and/or a marketing application may also be discussed. FDA will also provide its best judgment, at that time, of the pediatric studies that will be required for the drug product and whether their submission will be deferred until after approval. Agreements reached at the meeting on these matters will be recorded in minutes of the conference that will be taken by FDA in accordance with § 10.65 and provided to the sponsor. The minutes along with any other written material provided to the sponsor will serve as a permanent record of any agreements reached. Barring a significant scientific development that requires otherwise, studies conducted in accordance with the agreement shall be presumed to be sufficient in objective and design for the purpose of obtaining marketing approval for the drug.

(2) *"Pre-NDA" and "pre-BLA" meetings.* FDA has found that delays associated with the initial review of a marketing application may be reduced by exchanges of information about a proposed marketing application. The primary purpose of this kind of exchange is to uncover any major unresolved problems, to identify those studies that the sponsor is relying on as adequate and well-controlled to establish the drug's effectiveness, to identify the status of ongoing or needed studies adequate to assess pediatric safety and effectiveness, to acquaint FDA reviewers with the general information to be submitted in the marketing application (including technical information), to discuss appropriate methods for statistical analysis of the data, and to discuss the best approach to the presentation and formatting of data in the marketing application. Arrangements for such a meeting are to be initiated by the sponsor with the division responsible for review of the IND. To permit FDA to provide the sponsor with the most useful advice on preparing a marketing application, the sponsor should submit to FDA's reviewing division at least 1 month in advance of the meeting the following information:

(i) A brief summary of the clinical studies to be submitted in the application.

(ii) A proposed format for organizing the submission, including methods for presenting the data.

(iii) Information on the status of needed or ongoing pediatric studies.

(iv) Any other information for discussion at the meeting.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 63 FR 66669, Dec. 2, 1998; 67 FR 9586, Mar. 4, 2002]

§ 312.48 Dispute resolution.

(a) *General.* The Food and Drug Administration is committed to resolving differences between sponsors and FDA reviewing divisions with respect to requirements for IND's as quickly and amicably as possible through the cooperative exchange of information and views.

(b) *Administrative and procedural issues.* When administrative or procedural disputes arise, the sponsor should first attempt to resolve the matter with the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND, beginning with the consumer safety officer assigned to the application. If the dispute is not resolved, the sponsor may raise the matter with the person designated as ombudsman, whose function shall be to investigate what has happened and to facilitate a timely and equitable resolution. Appropriate issues to raise with the ombudsman include resolving difficulties in scheduling meetings and obtaining timely replies to inquiries. Further details on this procedure are contained in FDA Staff Manual Guide 4820.7 that is publicly available under FDA's public information regulations in part 20.

(c) *Scientific and medical disputes.* (1) When scientific or medical disputes arise during the drug investigation process, sponsors should discuss the matter directly with the responsible reviewing officials. If necessary, sponsors may request a meeting with the appropriate reviewing officials and management representatives in order to seek a resolution. Requests for such meetings shall be directed to the director of the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND. FDA will make every attempt to grant requests for meetings that involve important issues and that can be scheduled at mutually convenient times.

(2) The "end-of-Phase 2" and "pre-NDA" meetings described in § 312.47(b) will also provide a timely forum for discussing and resolving scientific and medical issues on which the sponsor disagrees with the agency.

(3) In requesting a meeting designed to resolve a scientific or medical dispute, applicants may suggest that FDA seek the advice of outside experts, in which case FDA may, in its discretion, invite to the meeting one or more of its advisory committee members or other consultants, as designated by the agency. Applicants may rely on, and may bring to any meeting, their own consultants. For major scientific and medical policy issues not resolved by informal meetings, FDA may refer the matter to one of its standing advisory committees for its consideration and recommendations.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990]

Subpart D—Responsibilities of Sponsors and Investigators

§ 312.50 General responsibilities of sponsors.

Sponsors are responsibile for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation(s), ensuring that the investigation(s) is conducted in accordance with the general investigational plan and protocols contained in the IND, maintaining an effective IND with respect to the investigations, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug. Additional specific responsibilities of sponsors are described elsewhere in this part.

§ 312.52 Transfer of obligations to a contract research organization.

(a) A sponsor may transfer responsibility for any or all of the obligations set forth in this part to a contract research organization. Any such transfer shall be described in writing. If not all obligations are transferred, the writing is required to describe each of the obligations being assumed by the contract research organization. If all obligations are transferred, a general statement that all obligations have been transferred is acceptable. Any obligation not covered by the written description shall be deemed not to have been transferred.

(b) A contract research organization that assumes any obligation of a sponsor shall comply with the specific regulations in this chapter applicable to this obligation and shall be subject to the same regulatory action as a sponsor for failure to comply with any obligation assumed under these regulations. Thus, all references to "sponsor" in this part apply to a contract research organization to the extent that it assumes one or more obligations of the sponsor.

§ 312.53 Selecting investigators and monitors.

(a) *Selecting investigators.* A sponsor shall select only investigators qualified by training and experience as appropriate experts to investigate the drug.

(b) *Control of drug.* A sponsor shall ship investigational new drugs only to investigators participating in the investigation.

(c) *Obtaining information from the investigator.* Before permitting an investigator to begin participation in an investigation, the sponsor shall obtain the following:

(1) A signed investigator statement (Form FDA-1572) containing:

(i) The name and address of the investigator;

(ii) The name and code number, if any, of the protocol(s) in the IND identifying the study(ies) to be conducted by the investigator;

(iii) The name and address of any medical school, hospital, or other research facility where the clinical investigation(s) will be conducted;

(iv) The name and address of any clinical laboratory facilities to be used in the study;

(v) The name and address of the IRB that is responsible for review and approval of the study(ies);

(vi) A commitment by the investigator that he or she:

(*a*) Will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, the rights, or welfare of subjects;

(*b*) Will comply with all requirements regarding the obligations of clinical investigators and all other pertinent requirements in this part;

(*c*) Will personally conduct or supervise the described investigation(s);

(*d*) Will inform any potential subjects that the drugs are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent (21 CFR part 50) and institutional review board review and approval (21 CFR part 56) are met;

(*e*) Will report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with § 312.64;

(*f*) Has read and understands the information in the investigator's brochure, including the potential risks and side effects of the drug; and

(*g*) Will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

(vii) A commitment by the investigator that, for an investigation subject to an institutional review requirement under part 56, an IRB that complies with the requirements of that part will be responsible for the initial and continuing review and approval of the clinical investigation and that the investigator will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to the human subjects.

(viii) A list of the names of the subinvestigators (e.g., research fellows, residents) who will be assisting the investigator in the conduct of the investigation(s).

(2) *Curriculum vitae.* A curriculum vitae or other statement of qualifications of the investigator showing the education, training, and experience that qualifies the investigator as an expert in the clinical investigation of the drug for the use under investigation.

(3) *Clinical protocol.* (i) For Phase 1 investigations, a general outline of the planned investigation including the estimated duration of the study and the maximum number of subjects that will be involved.

(ii) For Phase 2 or 3 investigations, an outline of the study protocol including an approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; the clinical uses to be investigated; characteristics of subjects by age, sex, and condition; the kind of clinical observations and laboratory tests to be conducted; the estimated duration of the study; and copies or a description of case report forms to be used.

(4) *Financial disclosure information.* Sufficient accurate financial information to allow the sponsor to submit complete and accurate certification or disclosure statements required under part 54 of this chapter. The sponsor shall obtain a commitment from the clinical investigator to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

(d) *Selecting monitors.* A sponsor shall select a monitor qualified by training and experience to monitor the progress of the investigation.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996; 63 FR 5252, Feb. 2, 1998; 67 FR 9586, Mar. 4, 2002]

§ 312.54 Emergency research under § 50.24 of this chapter.

(a) The sponsor shall monitor the progress of all investigations involving an exception from informed consent under § 50.24 of this chapter. When the sponsor receives from the IRB information concerning the public disclosures required by § 50.24(a)(7)(ii) and (a)(7)(iii) of this chapter, the sponsor promptly shall submit to the IND file and to Docket Number 95S-0158 in the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857, copies of the information that was disclosed, identified by the IND number.

(b) The sponsor also shall monitor such investigations to identify when an IRB determines that it cannot approve the research because it does not meet the criteria in the exception in § 50.24(a) of this chapter or because of other relevant ethical concerns. The sponsor promptly shall provide this information in writing to FDA, investigators who are asked to participate in this or a substantially equivalent clinical investigation, and other IRB's that are asked to review this or a substantially equivalent investigation.

[61 FR 51530, Oct. 2, 1996]

§ 312.55 Informing investigators.

(a) Before the investigation begins, a sponsor (other than a sponsor-investigator) shall give each participating clinical investigator an investigator brochure containing the information described in § 312.23(a)(5).

(b) The sponsor shall, as the overall investigation proceeds, keep each participating investigator informed of new observations discovered by or reported to the sponsor on the drug, particularly with respect to adverse effects and safe use. Such information may be distributed to investigators by means of periodically revised investigator brochures, reprints or published studies, reports or letters to clinical investigators, or other appropriate means. Important safety information is required to be relayed to investigators in accordance with § 312.32.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

§ 312.56 Review of ongoing investigations.

(a) The sponsor shall monitor the progress of all clinical investigations being conducted under its IND.

(b) A sponsor who discovers that an investigator is not complying with the signed agreement (Form FDA-1572), the general investigational plan, or the requirements of this part or other applicable parts shall promptly either secure compliance or discontinue shipments of the investigational new drug to the investigator and end the investigator's participation in the investigation. If the investigator's participation in the investigation is ended, the sponsor shall require that the investigator dispose of or return the investigational drug in accordance with the requirements of § 312.59 and shall notify FDA.

(c) The sponsor shall review and evaluate the evidence relating to the

safety and effectiveness of the drug as it is obtained from the investigator. The sponsors shall make such reports to FDA regarding information relevant to the safety of the drug as are required under §312.32. The sponsor shall make annual reports on the progress of the investigation in accordance with §312.33.

(d) A sponsor who determines that its investigational drug presents an unreasonable and significant risk to subjects shall discontinue those investigations that present the risk, notify FDA, all institutional review boards, and all investigators who have at any time participated in the investigation of the discontinuance, assure the disposition of all stocks of the drug outstanding as required by §312.59, and furnish FDA with a full report of the sponsor's actions. The sponsor shall discontinue the investigation as soon as possible, and in no event later than 5 working days after making the determination that the investigation should be discontinued. Upon request, FDA will confer with a sponsor on the need to discontinue an investigation.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

§312.57 Recordkeeping and record retention.

(a) A sponsor shall maintain adequate records showing the receipt, shipment, or other disposition of the investigational drug. These records are required to include, as appropriate, the name of the investigator to whom the drug is shipped, and the date, quantity, and batch or code mark of each such shipment.

(b) A sponsor shall maintain complete and accurate records showing any financial interest in §54.4(a)(3)(i), (a)(3)(ii), (a)(3)(iii), and (a)(3)(iv) of this chapter paid to clinical investigators by the sponsor of the covered study. A sponsor shall also maintain complete and accurate records concerning all other financial interests of investigators subject to part 54 of this chapter.

(c) A sponsor shall retain the records and reports required by this part for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified.

(d) A sponsor shall retain reserve samples of any test article and reference standard identified in, and used in any of the bioequivalence or bioavailability studies described in, §320.38 or §320.63 of this chapter, and release the reserve samples to FDA upon request, in accordance with, and for the period specified in §320.38.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 58 FR 25926, Apr. 28, 1993; 63 FR 5252, Feb. 2, 1998; 67 FR 9586, Mar. 4, 2002]

§312.58 Inspection of sponsor's records and reports.

(a) *FDA inspection.* A sponsor shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify any records and reports relating to a clinical investigation conducted under this part. Upon written request by FDA, the sponsor shall submit the records or reports (or copies of them) to FDA. The sponsor shall discontinue shipments of the drug to any investigator who has failed to maintain or make available records or reports of the investigation as required by this part.

(b) *Controlled substances.* If an investigational new drug is a substance listed in any schedule of the Controlled Substances Act (21 U.S.C. 801; 21 CFR part 1308), records concerning shipment, delivery, receipt, and disposition of the drug, which are required to be kept under this part or other applicable parts of this chapter shall, upon the request of a properly authorized employee of the Drug Enforcement Administration of the U.S. Department of Justice, be made available by the investigator or sponsor to whom the request is made, for inspection and copying. In addition, the sponsor shall assure that adequate precautions are taken, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§312.59 Disposition of unused supply of investigational drug.

The sponsor shall assure the return of all unused supplies of the investigational drug from each individual investigator whose participation in the investigation is discontinued or terminated. The sponsor may authorize alternative disposition of unused supplies of the investigational drug provided this alternative disposition does not expose humans to risks from the drug. The sponsor shall maintain written records of any disposition of the drug in accordance with §312.57.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

§312.60 General responsibilities of investigators.

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation. An investigator shall, in accordance with the provisions of part 50 of this chapter, obtain the informed consent of each human subject to whom the drug is administered, except as provided in §§50.23 or 50.24 of this chapter. Additional specific responsibilities of clinical investigators are set forth in this part and in parts 50 and 56 of this chapter.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51530, Oct. 2, 1996]

§312.61 Control of the investigational drug.

An investigator shall administer the drug only to subjects under the investigator's personal supervision or under the supervision of a subinvestigator responsible to the investigator. The investigator shall not supply the investigational drug to any person not authorized under this part to receive it.

§312.62 Investigator recordkeeping and record retention.

(a) *Disposition of drug.* An investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects. If the investigation is terminated, suspended, discontinued, or completed, the investigator shall return the unused supplies of the drug to the sponsor, or otherwise provide for disposition of the unused supplies of the drug under §312.59.

(b) *Case histories.* An investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

(c) *Record retention.* An investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996; 67 FR 9586, Mar. 4, 2002]

§312.64 Investigator reports.

(a) *Progress reports.* The investigator shall furnish all reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained. The sponsor is required under §312.33 to submit annual reports to FDA on the progress of the clinical investigations.

(b) *Safety reports.* An investigator shall promptly report to the sponsor any adverse effect that may reasonably be regarded as caused by, or probably

caused by, the drug. If the adverse effect is alarming, the investigator shall report the adverse effect immediately.

(c) *Final report.* An investigator shall provide the sponsor with an adequate report shortly after completion of the investigator's participation in the investigation.

(d) *Financial disclosure reports.* The clinical investigator shall provide the sponsor with sufficient accurate financial information to allow an applicant to submit complete and accurate certification or disclosure statements as required under part 54 of this chapter. The clinical investigator shall promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 5252, Feb. 2, 1998; 67 FR 9586, Mar. 4, 2002]

§ 312.66 Assurance of IRB review.

An investigator shall assure that an IRB that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of the proposed clinical study. The investigator shall also assure that he or she will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others, and that he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 67 FR 9586, Mar. 4, 2002]

§ 312.68 Inspection of investigator's records and reports.

An investigator shall upon request from any properly authorized officer or employee of FDA, at reasonable times, permit such officer or employee to have access to, and copy and verify any records or reports made by the investigator pursuant to § 312.62. The investigator is not required to divulge subject names unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual case studies, or do not represent actual results obtained.

§ 312.69 Handling of controlled substances.

If the investigational drug is subject to the Controlled Substances Act, the investigator shall take adequate precautions, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.70 Disqualification of a clinical investigator.

(a) If FDA has information indicating that an investigator (including a sponsor-investigator) has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has submitted to FDA or to the sponsor false information in any required report, the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research will furnish the investigator written notice of the matter complained of and offer the investigator an opportunity to explain the matter in writing, or, at the option of the investigator, in an informal conference. If an explanation is offered but not accepted by the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research, the investigator will be given an opportunity for a regulatory hearing under part 16 on the question of whether the investigator is entitled to receive investigational new drugs.

(b) After evaluating all available information, including any explanation presented by the investigator, if the Commissioner determines that the investigator has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has deliberately or repeatedly submitted false information to FDA or to the sponsor in any required report, the Commissioner will notify the investigator and the sponsor

of any investigation in which the investigator has been named as a participant that the investigator is not entitled to receive investigational drugs. The notification will provide a statement of basis for such determination.

(c) Each IND and each approved application submitted under part 314 containing data reported by an investigator who has been determined to be ineligible to receive investigational drugs will be examined to determine whether the investigator has submitted unreliable data that are essential to the continuation of the investigation or essential to the approval of any marketing application.

(d) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the data remaining are inadequate to support a conclusion that it is reasonably safe to continue the investigation, the Commissioner will notify the sponsor who shall have an opportunity for a regulatory hearing under part 16. If a danger to the public health exists, however, the Commissioner shall terminate the IND immediately and notify the sponsor of the determination. In such case, the sponsor shall have an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be reinstated.

(e) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the continued approval of the drug product for which the data were submitted cannot be justified, the Commissioner will proceed to withdraw approval of the drug product in accordance with the applicable provisions of the act.

(f) An investigator who has been determined to be ineligible to receive investigational drugs may be reinstated as eligible when the Commissioner determines that the investigator has presented adequate assurances that the investigator will employ investigatioal drugs solely in compliance with the provisions of this part and of parts 50 and 56.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 62 FR 46876, Sept. 5, 1997; 67 FR 9586, Mar. 4, 2002]

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

AUTHORITY: 21 U.S.C. 351, 352, 353, 355, 371; 42 U.S.C. 262.

SOURCE: 53 FR 41523, Oct. 21, 1988, unless otherwise noted.

§ 312.80 Purpose.

The purpose of this section is to establish procedures designed to expedite the development, evaluation, and marketing of new therapies intended to treat persons with life-threatening and severely-debilitating illnesses, especially where no satisfactory alternative therapy exists. As stated §314.105(c) of this chapter, while the statutory standards of safety and effectiveness apply to all drugs, the many kinds of drugs that are subject to them, and the wide range of uses for those drugs, demand flexibility in applying the standards. The Food and Drug Administration (FDA) has determined that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness. These procedures reflect the recognition that physicians and patients are generally willing to accept greater risks or side effects from products that treat life-threatening and severely-debilitating illnesses, than they would accept from products that treat less serious illnesses. These procedures also reflect the recognition that the benefits of the drug need to be evaluated in light of the severity of the disease being treated. The procedure outlined in this section should be interpreted consistent with that purpose.

§ 312.81 Scope.

This section applies to new drug and biological products that are being studied for their safety and effectiveness in treating life-threatening or severely-debilitating diseases.

(a) For purposes of this section, the term "life-threatening" means:

(1) Diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted; and

(2) Diseases or conditions with potentially fatal outcomes, where the end point of clinical trial analysis is survival.

(b) For purposes of this section, the term "severely debilitating" means diseases or conditions that cause major irreversible morbidity.

(c) Sponsors are encouraged to consult with FDA on the applicability of these procedures to specific products.

[53 FR 41523, Oct. 21, 1988, as amended at 64 FR 401, Jan. 5, 1999]

§ 312.82 Early consultation.

For products intended to treat life-threatening or severely-debilitating illnesses, sponsors may request to meet with FDA-reviewing officials early in the drug development process to review and reach agreement on the design of necessary preclinical and clinical studies. Where appropriate, FDA will invite to such meetings one or more outside expert scientific consultants or advisory committee members. To the extent FDA resources permit, agency reviewing officials will honor requests for such meetings

(a) *Pre-investigational new drug (IND) meetings.* Prior to the submission of the initial IND, the sponsor may request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of animal studies needed to initiate human testing. The meeting may also provide an opportunity for discussing the scope and design of phase 1 testing, plans for studying the drug product in pediatric populations, and the best approach for presentation and formatting of data in the IND.

(b) *End-of-phase 1 meetings.* When data from phase 1 clinical testing are available, the sponsor may again request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of phase 2 controlled clinical trials, with the goal that such testing will be adequate to provide sufficient data on the drug's safety and effectiveness to support a decision on its approvability for marketing, and to discuss the need for, as well as the design and timing of, studies of the drug in pediatric patients. For drugs for life-threatening diseases, FDA will provide its best judgment, at that time, whether pediatric studies will be required and whether their submission will be deferred until after approval. The procedures outlined in §312.47(b)(1) with respect to end-of-phase 2 conferences, including documentation of agreements reached, would also be used for end-of-phase 1 meetings.

[53 FR 41523, Oct. 21, 1988, as amended at 63 FR 66669, Dec. 2, 1998]

§ 312.83 Treatment protocols.

If the preliminary analysis of phase 2 test results appears promising, FDA may ask the sponsor to submit a treatment protocol to be reviewed under the procedures and criteria listed in §§312.34 and 312.35. Such a treatment protocol, if requested and granted, would normally remain in effect while the complete data necessary for a marketing application are being assembled by the sponsor and reviewed by FDA (unless grounds exist for clinical hold of ongoing protocols, as provided in §312.42(b)(3)(ii)).

§ 312.84 Risk-benefit analysis in review of marketing applications for drugs to treat life-threatening and severely-debilitating illnesses.

(a) FDA's application of the statutory standards for marketing approval shall recognize the need for a medical risk-benefit judgment in making the final decision on approvability. As part of this evaluation, consistent with the statement of purpose in §312.80, FDA will consider whether the benefits of the drug outweigh the known and potential risks of the drug and the need to answer remaining questions about risks and benefits of the drug, taking into consideration the severity of the disease and the absence of satisfactory alternative therapy.

(b) In making decisions on whether to grant marketing approval for products that have been the subject of an end-of-phase 1 meeting under §312.82, FDA will usually seek the advice of outside expert scientific consultants or advisory committees. Upon the filing of such a marketing application under §314.101 or part 601 of this chapter, FDA will notify the members of the relevant standing advisory committee of the application's filing and its availability for review.

(c) If FDA concludes that the data presented are not sufficient for marketing approval, FDA will issue (for a drug) a not approvable letter pursuant to §314.120 of this chapter, or (for a biologic) a deficiencies letter consistent with the biological product licensing procedures. Such letter, in describing the deficiencies in the application, will address why the results of the research design agreed to under §312.82, or in subsequent meetings, have not provided sufficient evidence for marketing approval. Such letter will also describe any recommendations made by the advisory committee regarding the application.

(d) Marketing applications submitted under the procedures contained in this section will be subject to the requirements and procedures contained in part 314 or part 600 of this chapter, as well as those in this subpart.

§ 312.85 Phase 4 studies.

Concurrent with marketing approval, FDA may seek agreement from the sponsor to conduct certain post-marketing (phase 4) studies to delineate additional information about the drug's risks, benefits, and optimal use. These studies could include, but would not be limited to, studying different doses or schedules of administration than were used in phase 2 studies, use of the drug in other patient populations or other stages of the disease, or use of the drug over a longer period of time.

§ 312.86 Focused FDA regulatory research.

At the discretion of the agency, FDA may undertake focused regulatory research on critical rate-limiting aspects of the preclinical, chemical/manufacturing, and clinical phases of drug development and evaluation. When initiated, FDA will undertake such research efforts as a means for meeting a public health need in facilitating the development of therapies to treat life-threatening or severely debilitating illnesses.

§ 312.87 Active monitoring of conduct and evaluation of clinical trials.

For drugs covered under this section, the Commissioner and other agency officials will monitor the progress of the conduct and evaluation of clinical trials and be involved in facilitating their appropriate progress.

§ 312.88 Safeguards for patient safety.

All of the safeguards incorporated within parts 50, 56, 312, 314, and 600 of this chapter designed to ensure the safety of clinical testing and the safety of products following marketing approval apply to drugs covered by this section. This includes the requirements for informed consent (part 50 of this chapter) and institutional review boards (part 56 of this chapter). These safeguards further include the review of animal studies prior to initial human testing (§312.23), and the monitoring of adverse drug experiences through the requirements of IND safety reports (§312.32), safety update reports during agency review of a marketing application (§314.50 of this chapter), and postmarketing adverse reaction reporting (§314.80 of this chapter).

Subpart F—Miscellaneous

§ 312.110 Import and export requirements.

(a) *Imports.* An investigational new drug offered for import into the United States complies with the requirements of this part if it is subject to an IND that is in effect for it under §312.40 and: (1) The consignee in the United States is the sponsor of the IND; (2) the consignee is a qualified investigator named in the IND; or (3) the consignee is the domestic agent of a foreign sponsor, is responsible for the control and distribution of the investigational drug, and the IND identifies the consignee and describes what, if any, actions the consignee will take with respect to the investigational drug.

(b) *Exports.* An investigational new drug intended for export from the United States complies with the requirements of this part as follows:

(1) If an IND is in effect for the drug under §312.40 and each person who receives the drug is an investigator named in the application; or

(2) If FDA authorizes shipment of the drug for use in a clinical investigation. Authorization may be obtained as follows:

(i) Through submission to the International Affairs Staff (HFY–50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a written request from the person that seeks to export the drug. A request must provide adequate information about the drug to satisfy FDA that the drug is appropriate for the proposed investigational use in humans, that the drug will be used for investigational purposes only, and that the drug may be legally used by that consignee in the importing country for the proposed investigational use. The request shall specify the quantity of the drug to be shipped per shipment and the frequency of expected shipments. If FDA authorizes exportation under this paragraph, the agency shall concurrently notify the government of the importing country of such authorization.

(ii) Through submission to the International Affairs Staff (HFY–50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a formal request from an authorized official of the government of the country to which the drug is proposed to be shipped. A request must specify that the foreign government has adequate information about the drug and the proposed investigational use, that the drug will be used for investigational purposes only, and that the foreign government is satisfied that the drug may legally be used by the intended consignee in that country. Such a request shall specify the quantity of drug to be shipped per shipment and the frequency of expected shipments.

(iii) Authorization to export an investigational drug under paragraph (b)(2)(i) or (ii) of this section may be revoked by FDA if the agency finds that the conditions underlying its authorization are not longer met.

(3) This paragraph applies only where the drug is to be used for the purpose of clinical investigation.

(4) This paragraph does not apply to the export of new drugs (including biological products, antibiotic drugs, and insulin) approved or authorized for export under section 802 of the act (21 U.S.C. 382) or section 351(h)(1)(A) of the Public Health Service Act (42 U.S.C. 262(h)(1)(A)).

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 64 FR 401, Jan. 5, 1999; 67 FR 9586, Mar. 4, 2002]

§ 312.120 Foreign clinical studies not conducted under an IND.

(a) *Introduction.* This section describes the criteria for acceptance by FDA of foreign clinical studies not conducted under an IND. In general, FDA accepts such studies provided they are well designed, well conducted, performed by qualified investigators, and conducted in accordance with ethical principles acceptable to the world community. Studies meeting these criteria may be utilized to support clinical investigations in the United States and/or marketing approval. Marketing approval of a new drug based solely on foreign clinical data is governed by §314.106.

(b) *Data submissions.* A sponsor who wishes to rely on a foreign clinical study to support an IND or to support an application for marketing approval shall submit to FDA the following information:

(1) A description of the investigator's qualifications;

(2) A description of the research facilities;

(3) A detailed summary of the protocol and results of the study, and, should FDA request, case records maintained by the investigator or additional background data such as hospital or other institutional records;

(4) A description of the drug substance and drug product used in the study, including a description of components, formulation, specifications, and bioavailability of the specific drug product used in the clinical study, if available; and

(5) If the study is intended to support the effectiveness of a drug product, information showing that the study is adequate and well controlled under §314.126.

(c) *Conformance with ethical principles.* (1) Foreign clinical research is required to have been conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" (see paragraph (c)(4) of this section) or the laws and regulations of the country in which the research was conducted, whichever represents the greater protection of the individual.

(2) For each foreign clinical study submitted under this section, the sponsor shall explain how the research conformed to the ethical principles contained in the "Declaration of Helsinki" or the foreign country's standards, whichever were used. If the foreign country's standards were used, the sponsor shall explain in detail how those standards differ from the "Declaration of Helsinki" and how they offer greater protection.

(3) When the research has been approved by an independent review committee, the sponsor shall submit to FDA documentation of such review and approval, including the names and qualifications of the members of the committee. In this regard, a "review committee" means a committee composed of scientists and, where practicable, individuals who are otherwise qualified (e.g., other health professionals or laymen). The investigator may not vote on any aspect of the review of his or her protocol by a review committee.

(4) The "Declaration of Helsinki" states as follows:

RECOMMENDATIONS GUIDING PHYSICIANS IN BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded

by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient—including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers—either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 56 FR 22113, May 14, 1991; 64 FR 401, Jan. 5, 1999; 67 FR 9586, Mar. 4, 2002]

§312.130 Availability for public disclosure of data and information in an IND.

(a) The existence of an investigational new drug application will not be disclosed by FDA unless it has previously been publicly disclosed or acknowledged.

(b) The availability for public disclosure of all data and information in an investigational new drug application for a new drug will be handled in accordance with the provisions established in §314.430 for the confidentiality of data and information in applications submitted in part 314. The availability for public disclosure of all data and information in an investigational new drug application for a biological product will be governed by the provisions of §§601.50 and 601.51.

(c) Notwithstanding the provisions of §314.430, FDA shall disclose upon request to an individual to whom an investigational new drug has been given a copy of any IND safety report relating to the use in the individual.

(d) The availability of information required to be publicly disclosed for investigations involving an exception from informed consent under §50.24 of this chapter will be handled as follows: Persons wishing to request the publicly disclosable information in the IND that was required to be filed in Docket Number 95S-0158 in the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857, shall submit a request under the Freedom of Information Act.

[52 FR 8831, Mar. 19, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988, as amended at 61 FR 51630, Oct. 2, 1996; 64 FR 401, Jan. 5, 1999]

§312.140 Address for correspondence.

(a) Except as provided in paragraph (b) of this section, a sponsor shall send an initial IND submission to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, Park Bldg., Rm. 214, 12420 Parklawn Dr., Rockville, MD 20852. On receiving the IND, FDA will inform the sponsor which one of the divisions in the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research is responsible for the IND. Amendments, reports, and other correspondence relating to matters covered by the IND should be directed to the appropriate division. The outside wrapper of each submission shall state what is contained in the submission, for example, "IND Application", "Protocol Amendment", etc.

(b) Applications for the products listed below should be submitted to the Division of Biological Investigational New Drugs (HFB-230), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892. (1) Products subject to the licensing provisions of the Public Health Service Act of July 1, 1944 (58 Stat. 682, as amended (42 U.S.C. 201 *et seq.*)) or subject to part 600; (2) ingredients packaged together with containers intended for the collection, processing, or storage of blood or blood components; (3) urokinase products; (4) plasma volume expanders and hydroxyethyl starch for leukapheresis; and (5) coupled antibodies, i.e., products that consist of an antibody component coupled with a drug or radionuclide component in which both components provide a pharmacological effect but the biological component determines the site of action.

(c) All correspondence relating to biological products for human use which are also radioactive drugs shall be submitted to the Division of Oncology and Radiopharmaceutical Drug Products (HFD-150), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, except that applications for coupled antibodies shall be submitted in accordance with paragraph (b) of this section.

(d) All correspondence relating to export of an investigational drug under §312.110(b)(2) shall be submitted to the International Affairs Staff (HFY-50), Office of Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 67 FR 9586, Mar. 4, 2002]

§312.145 Guidance documents.

(a) FDA has made available guidance documents under §10.115 of this chapter to help you to comply with certain requirements of this part.

(b) The Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) maintain lists of guidance documents that apply to the centers' regulations. The lists are maintained on

the Internet and are published annually in the FEDERAL REGISTER. A request for a copy of the CDER list should be directed to the Office of Training and Communications, Division of Communications Management, Drug Information Branch (HFD-210), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. A request for a copy of the CBER list should be directed to the Office of Communication, Training, and Manufacturers Assistance (HFM-40), Center for Biologics Evaluation and Research, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852-1448.

[65 FR 56479, Sept. 19, 2000]

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or In Vitro Tests

§ 312.160 Drugs for investigational use in laboratory research animals or in vitro tests.

(a) *Authorization to ship.* (1)(i) A person may ship a drug intended solely for tests in vitro or in animals used only for laboratory research purposes if it is labeled as follows:

CAUTION: Contains a new drug for investigational use only in laboratory research animals, or for tests in vitro. Not for use in humans.

(ii) A person may ship a biological product for investigational in vitro diagnostic use that is listed in § 312.2(b)(2)(ii) if it is labeled as follows:

CAUTION: Contains a biological product for investigational in vitro diagnostic tests only.

(2) A person shipping a drug under paragraph (a) of this section shall use due diligence to assure that the consignee is regularly engaged in conducting such tests and that the shipment of the new drug will actually be used for tests in vitro or in animals used only for laboratory research.

(3) A person who ships a drug under paragraph (a) of this section shall maintain adequate records showing the name and post office address of the expert to whom the drug is shipped and the date, quantity, and batch or code mark of each shipment and delivery. Records of shipments under paragraph (a)(1)(i) of this section are to be maintained for a period of 2 years after the shipment. Records and reports of data and shipments under paragraph (a)(1)(ii) of this section are to be maintained in accordance with § 312.57(b). The person who ships the drug shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify records required to be maintained under this section.

(b) *Termination of authorization to ship.* FDA may terminate authorization to ship a drug under this section if it finds that:

(1) The sponsor of the investigation has failed to comply with any of the conditions for shipment established under this section; or

(2) The continuance of the investigation is unsafe or otherwise contrary to the public interest or the drug is used for purposes other than bona fide scientific investigation. FDA will notify the person shipping the drug of its finding and invite immediate correction. If correction is not immediately made, the person shall have an opportunity for a regulatory hearing before FDA pursuant to part 16.

(c) *Disposition of unused drug.* The person who ships the drug under paragraph (a) of this section shall assure the return of all unused supplies of the drug from individual investigators whenever the investigation discontinues or the investigation is terminated. The person who ships the drug may authorize in writing alternative disposition of unused supplies of the drug provided this alternative disposition does not expose humans to risks from the drug, either directly or indirectly (e.g., through food-producing animals). The shipper shall maintain records of any alternative disposition.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988; 67 FR 9586, Mar. 4, 2002]

(n) *Assent* means a child's affirmative agreement to participate in a clinical investigation. Mere failure to object may not, absent affirmative agreement, be construed as assent.

(o) *Children* means persons who have not attained the legal age for consent to treatments or procedures involved in clinical investigations, under the applicable law of the jurisdiction in which the clinical investigation will be conducted.

(p) *Parent* means a child's biological or adoptive parent.

(q) *Ward* means a child who is placed in the legal custody of the State or other agency, institution, or entity, consistent with applicable Federal, State, or local law.

(r) *Permission* means the agreement of parent(s) or guardian to the participation of their child or ward in a clinical investigation. Permission must be obtained in compliance with subpart B of this part and must include the elements of informed consent described in §50.25.

(s) *Guardian* means an individual who is authorized under applicable State or local law to consent on behalf of a child to general medical care when general medical care includes participation in research. For purposes of subpart D of this part, a guardian also means an individual who is authorized to consent on behalf of a child to participate in research.

[45 FR 36390, May 30, 1980, as amended at 46 FR 8950, Jan. 27, 1981; 54 FR 9038, Mar. 3, 1989; 56 FR 28028, June 18, 1991; 61 FR 51528, Oct. 2, 1996; 62 FR 39440, July 23, 1997; 64 FR 399, Jan. 5, 1999; 64 FR 56448, Oct. 20, 1999; 66 FR 20597, Apr. 24, 2001]

Subpart B—Informed Consent of Human Subjects

SOURCE: 46 FR 8951, Jan. 27, 1981, unless otherwise noted.

§50.20 General requirements for informed consent.

Except as provided in §§50.23 and 50.24, no investigator may involve a human being as a subject in research covered by these regulations unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative. No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution, or its agents from liability for negligence.

[46 FR 8951, Jan. 27, 1981, as amended at 64 FR 10942, Mar. 8, 1999]

§50.23 Exception from general requirements.

(a) The obtaining of informed consent shall be deemed feasible unless, before use of the test article (except as provided in paragraph (b) of this section), both the investigator and a physician who is not otherwise participating in the clinical investigation certify in writing all of the following:

(1) The human subject is confronted by a life-threatening situation necessitating the use of the test article.

(2) Informed consent cannot be obtained from the subject because of an inability to communicate with, or obtain legally effective consent from, the subject.

(3) Time is not sufficient to obtain consent from the subject's legal representative.

(4) There is available no alternative method of approved or generally recognized therapy that provides an equal or greater likelihood of saving the life of the subject.

(b) If immediate use of the test article is, in the investigator's opinion, required to preserve the life of the subject, and time is not sufficient to obtain the independent determination required in paragraph (a) of this section in advance of using the test article, the determinations of the clinical investigator shall be made and, within 5 working days after the use of the article, be

reviewed and evaluated in writing by a physician who is not participating in the clinical investigation.

(c) The documentation required in paragraph (a) or (b) of this section shall be submitted to the IRB within 5 working days after the use of the test article.

(d)(1) Under 10 U.S.C. 1107(f) the President may waive the prior consent requirement for the administration of an investigational new drug to a member of the armed forces in connection with the member's participation in a particular military operation. The statute specifies that only the President may waive informed consent in this connection and the President may grant such a waiver only if the President determines in writing that obtaining consent: Is not feasible; is contrary to the best interests of the military member; or is not in the interests of national security. The statute further provides that in making a determination to waive prior informed consent on the ground that it is not feasible or the ground that it is contrary to the best interests of the military members involved, the President shall apply the standards and criteria that are set forth in the relevant FDA regulations for a waiver of the prior informed consent requirements of section 505(i)(4) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355(i)(4)). Before such a determination may be made that obtaining informed consent from military personnel prior to the use of an investigational drug (including an antibiotic or biological product) in a specific protocol under an investigational new drug application (IND) sponsored by the Department of Defense (DOD) and limited to specific military personnel involved in a particular military operation is not feasible or is contrary to the best interests of the military members involved the Secretary of Defense must first request such a determination from the President, and certify and document to the President that the following standards and criteria contained in paragraphs (d)(1) through (d)(4) of this section have been met.

(i) The extent and strength of evidence of the safety and effectiveness of the investigational new drug in relation to the medical risk that could be encountered during the military operation supports the drug's administration under an IND.

(ii) The military operation presents a substantial risk that military personnel may be subject to a chemical, biological, nuclear, or other exposure likely to produce death or serious or life-threatening injury or illness.

(iii) There is no available satisfactory alternative therapeutic or preventive treatment in relation to the intended use of the investigational new drug.

(iv) Conditioning use of the investigational new drug on the voluntary participation of each member could significantly risk the safety and health of any individual member who would decline its use, the safety of other military personnel, and the accomplishment of the military mission.

(v) A duly constituted institutional review board (IRB) established and operated in accordance with the requirements of paragraphs (d)(2) and (d)(3) of this section, responsible for review of the study, has reviewed and approved the investigational new drug protocol and the administration of the investigational new drug without informed consent. DOD's request is to include the documentation required by §56.115(a)(2) of this chapter.

(vi) DOD has explained:

(A) The context in which the investigational drug will be administered, e.g., the setting or whether it will be self-administered or it will be administered by a health professional;

(B) The nature of the disease or condition for which the preventive or therapeutic treatment is intended; and

(C) To the extent there are existing data or information available, information on conditions that could alter the effects of the investigational drug.

(vii) DOD's recordkeeping system is capable of tracking and will be used to track the proposed treatment from supplier to the individual recipient.

(viii) Each member involved in the military operation will be given, prior to the administration of the investigational new drug, a specific written information sheet (including information required by 10 U.S.C. 1107(d)) concerning the investigational new drug, the risks and benefits of its use, potential side effects, and other pertinent information about the appropriate use of the product.

(ix) Medical records of members involved in the military operation will accurately document the receipt by members of the notification required by paragraph (d)(1)(viii) of this section.

(x) Medical records of members involved in the military operation will accurately document the receipt by members of any investigational new drugs in accordance with FDA regulations including part 312 of this chapter.

(xi) DOD will provide adequate followup to assess whether there are beneficial or adverse health consequences that result from the use of the investigational product.

(xii) DOD is pursuing drug development, including a time line, and marketing approval with due diligence.

(xiii) FDA has concluded that the investigational new drug protocol may proceed subject to a decision by the President on the informed consent waiver request.

(xiv) DOD will provide training to the appropriate medical personnel and potential recipients on the specific investigational new drug to be administered prior to its use.

(xv) DOD has stated and justified the time period for which the waiver is needed, not to exceed one year, unless separately renewed under these standards and criteria.

(xvi) DOD shall have a continuing obligation to report to the FDA and to the President any changed circumstances relating to these standards and criteria (including the time period referred to in paragraph (d)(1)(xv) of this section) or that otherwise might affect the determination to use an investigational new drug without informed consent.

(xvii) DOD is to provide public notice as soon as practicable and consistent with classification requirements through notice in the FEDERAL REGISTER describing each waiver of informed consent determination, a summary of the most updated scientific information on the products used, and other pertinent information.

(xviii) Use of the investigational drug without informed consent otherwise conforms with applicable law.

(2) The duly constituted institutional review board, described in paragraph (d)(1)(v) of this section, must include at least 3 nonaffiliated members who shall not be employees or officers of the Federal Government (other than for purposes of membership on the IRB) and shall be required to obtain any necessary security clearances. This IRB shall review the proposed IND protocol at a convened meeting at which a majority of the members are present including at least one member whose primary concerns are in nonscientific areas and, if feasible, including a majority of the nonaffiliated members. The information required by §56.115(a)(2) of this chapter is to be provided to the Secretary of Defense for further review.

(3) The duly constituted institutional review board, described in paragraph (d)(1)(v) of this section, must review and approve:

(i) The required information sheet;

(ii) The adequacy of the plan to disseminate information, including distribution of the information sheet to potential recipients, on the investigational product (e.g., in forms other than written);

(iii) The adequacy of the information and plans for its dissemination to health care providers, including potential side effects, contraindications, potential interactions, and other pertinent considerations; and

(iv) An informed consent form as required by part 50 of this chapter, in those circumstances in which DOD determines that informed consent may be obtained from some or all personnel involved.

(4) DOD is to submit to FDA summaries of institutional review board meetings at which the proposed protocol has been reviewed.

(5) Nothing in these criteria or standards is intended to preempt or limit FDA's and DOD's authority or obligations under applicable statutes and regulations.

[46 FR 8951, Jan. 27, 1981, as amended at 55 FR 52817, Dec. 21, 1990; 64 FR 399, Jan. 5, 1999; 64 FR 54188, Oct. 5, 1999]

§ 50.24 Exception from informed consent requirements for emergency research.

(a) The IRB responsible for the review, approval, and continuing review of the clinical investigation described in this section may approve that investigation without requiring that informed consent of all research subjects be obtained if the IRB (with the concurrence of a licensed physician who is a member of or consultant to the IRB and who is not otherwise participating in the clinical investigation) finds and documents each of the following:

(1) The human subjects are in a life-threatening situation, available treatments are unproven or unsatisfactory, and the collection of valid scientific evidence, which may include evidence obtained through randomized placebo-controlled investigations, is necessary to determine the safety and effectiveness of particular interventions.

(2) Obtaining informed consent is not feasible because:

(i) The subjects will not be able to give their informed consent as a result of their medical condition;

(ii) The intervention under investigation must be administered before consent from the subjects' legally authorized representatives is feasible; and

(iii) There is no reasonable way to identify prospectively the individuals likely to become eligible for participation in the clinical investigation.

(3) Participation in the research holds out the prospect of direct benefit to the subjects because:

(i) Subjects are facing a life-threatening situation that necessitates intervention;

(ii) Appropriate animal and other preclinical studies have been conducted, and the information derived from those studies and related evidence support the potential for the intervention to provide a direct benefit to the individual subjects; and

(iii) Risks associated with the investigation are reasonable in relation to what is known about the medical condition of the potential class of subjects, the risks and benefits of standard therapy, if any, and what is known about the risks and benefits of the proposed intervention or activity.

(4) The clinical investigation could not practicably be carried out without the waiver.

(5) The proposed investigational plan defines the length of the potential therapeutic window based on scientific evidence, and the investigator has committed to attempting to contact a legally authorized representative for each subject within that window of time and, if feasible, to asking the legally authorized representative contacted for consent within that window rather than proceeding without consent. The investigator will summarize efforts made to contact legally authorized representatives and make this information available to the IRB at the time of continuing review.

(6) The IRB has reviewed and approved informed consent procedures and an informed consent document consistent with §50.25. These procedures and the informed consent document are to be used with subjects or their legally authorized representatives in situations where use of such procedures and documents is feasible. The IRB has reviewed and approved procedures and information to be used when providing an opportunity for a family member to object to a subject's participation in the clinical investigation consistent with paragraph (a)(7)(v) of this section.

(7) Additional protections of the rights and welfare of the subjects will be provided, including, at least:

(i) Consultation (including, where appropriate, consultation carried out by the IRB) with representatives of the communities in which the clinical investigation will be conducted and from which the subjects will be drawn;

(ii) Public disclosure to the communities in which the clinical investigation will be conducted and from which the subjects will be drawn, prior to initiation of the clinical investigation, of plans for the investigation and its risks and expected benefits;

(iii) Public disclosure of sufficient information following completion of the clinical investigation to apprise the community and researchers of the study, including the demographic characteristics of the research population, and its results;

(iv) Establishment of an independent data monitoring committee to exercise oversight of the clinical investigation; and

(v) If obtaining informed consent is not feasible and a legally authorized representative is not reasonably available, the investigator has committed, if feasible, to attempting to contact within the therapeutic window the subject's family member who is not a legally authorized representative, and asking whether he or she objects to the subject's participation in the clinical investigation. The investigator will summarize efforts made to contact family members and make this information available to the IRB at the time of continuing review.

(b) The IRB is responsible for ensuring that procedures are in place to inform, at the earliest feasible opportunity, each subject, or if the subject remains incapacitated, a legally authorized representative of the subject, or if such a representative is not reasonably available, a family member, of the subject's inclusion in the clinical investigation, the details of the investigation and other information contained in the informed consent document. The IRB shall also ensure that there is a procedure to inform the subject, or if the subject remains incapacitated, a legally authorized representative of the subject, or if such a representative is not reasonably available, a family member, that he or she may discontinue the subject's participation at any time without penalty or loss of benefits to which the subject is otherwise entitled. If a legally authorized representative or family member is told about the clinical investigation and the subject's condition improves, the subject is also to be informed as soon as feasible. If a subject is entered into a clinical investigation with waived consent and the subject dies before a legally authorized representative or family member can be contacted, information about the clinical investigation is to be provided to the subject's legally authorized representative or family member, if feasible.

(c) The IRB determinations required by paragraph (a) of this section and the documentation required by paragraph (e) of this section are to be retained by the IRB for at least 3 years after completion of the clinical investigation, and the records shall be accessible for inspection and copying by FDA in accordance with §56.115(b) of this chapter.

(d) Protocols involving an exception to the informed consent requirement under this section must be performed under a separate investigational new drug application (IND) or investigational device exemption (IDE) that clearly identifies such protocols as protocols that may include subjects who are unable to consent. The submission of those protocols in a separate IND/IDE is required even if an IND for the same drug product or an IDE for the same device already exists. Applications for investigations under this section may not be submitted as amendments under §§312.30 or 812.35 of this chapter.

(e) If an IRB determines that it cannot approve a clinical investigation because the investigation does not meet the criteria in the exception provided under paragraph (a) of this section or because of other relevant ethical concerns, the IRB must document its findings and provide these findings promptly in writing to the clinical investigator and to the sponsor of the clinical investigation. The sponsor of the clinical investigation must promptly disclose this information to FDA and to the sponsor's clinical investigators who are participating or are asked to participate in this or a substantially equivalent clinical investigation of the sponsor, and to other IRB's that have been, or are, asked to review this or a substantially equivalent investigation by that sponsor.

[61 FR 51528, Oct. 2, 1996]

§ 50.25 Elements of informed consent.

(a) *Basic elements of informed consent.* In seeking informed consent, the following information shall be provided to each subject:

(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental.

(2) A description of any reasonably foreseeable risks or discomforts to the subject.

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research.

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the Food and Drug Administration may inspect the records.

(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained.

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject.

(8) A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(b) *Additional elements of informed consent.* When appropriate, one or more of the following elements of information shall also be provided to each subject:

(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable.

(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent.

(3) Any additional costs to the subject that may result from participation in the research.

(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.

(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.

(6) The approximate number of subjects involved in the study.

(c) The informed consent requirements in these regulations are not intended to preempt any applicable Federal, State, or local laws which require additional information to be disclosed for informed consent to be legally effective.

(d) Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable Federal, State, or local law.

§ 50.27 Documentation of informed consent.

(a) Except as provided in § 56.109(c), informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject or the subject's legally authorized representative at the time of consent. A copy shall be given to the person signing the form.

(b) Except as provided in § 56.109(c), the consent form may be either of the following:

(1) A written consent document that embodies the elements of informed consent required by § 50.25. This form may be read to the subject or the subject's legally authorized representative, but, in any event, the investigator shall give either the subject or the representative adequate opportunity to read it before it is signed.

(2) A *short form* written consent document stating that the elements of informed consent required by § 50.25 have been presented orally to the subject or the subject's legally authorized representative. When this method is used, there shall be a witness to the oral presentation. Also, the IRB shall approve a written summary of what is to be said to the subject or the representative. Only the short form itself is to be signed by the subject or the representative. However, the witness shall

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)

Form Approved: OMB No. 0910-0014.
Expiration Date: January 31, 2006
See OMB Statement on Reverse.

NOTE: No drug may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40).

1. NAME OF SPONSOR

2. DATE OF SUBMISSION

3. ADDRESS *(Number, Street, City, State and Zip Code)*

4. TELEPHONE NUMBER *(Include Area Code)*

5. NAME(S) OF DRUG *(Include all available names: Trade, Generic, Chemical, Code)*

6. IND NUMBER *(If previously assigned)*

7. INDICATION(S) *(Covered by this submission)*

8. PHASE(S) OF CLINICAL INVESTIGATION TO BE CONDUCTED: ☐ PHASE 1 ☐ PHASE 2 ☐ PHASE 3 ☐ OTHER _____ *(Specify)*

9. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS (21 CFR Part 312), NEW DRUG OR ANTIBIOTIC APPLICATIONS *(21 CFR Part 314)*, DRUG MASTER FILES *(21 CFR Part 314.420)*, AND PRODUCT LICENSE APPLICATIONS (21 CFR Part 601) REFERRED TO IN THIS APPLICATION.

10. ***IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.***

SERIAL NUMBER
— — — —

11. THIS SUBMISSION CONTAINS THE FOLLOWING: *(Check all that apply)*

☐ INITIAL INVESTIGATIONAL NEW DRUG APPLICATION (IND)
☐ RESPONSE TO CLINICAL HOLD

PROTOCOL AMENDMENT(S):
☐ NEW PROTOCOL
☐ CHANGE IN PROTOCOL
☐ NEW INVESTIGATOR

INFORMATION AMENDMENT(S):
☐ CHEMISTRY/MICROBIOLOGY
☐ PHARMACOLOGY/TOXICOLOGY
☐ CLINICAL

IND SAFETY REPORT(S):
☐ INITIAL WRITTEN REPORT
☐ FOLLOW-UP TO A WRITTEN REPORT

☐ RESPONSE TO FDA REQUEST FOR INFORMATION
☐ ANNUAL REPORT
☐ GENERAL CORRESPONDENCE
☐ REQUEST FOR REINSTATEMENT OF IND THAT IS WITHDRAWN, INACTIVATED, TERMINATED OR DISCONTINUED
☐ OTHER _____ *(Specify)*

CHECK ONLY IF APPLICABLE

JUSTIFICATION STATEMENT MUST BE SUBMITTED WITH APPLICATION FOR ANY CHECKED BELOW. REFER TO THE CITED CFR SECTION FOR FURTHER INFORMATION.

☐ TREATMENT IND 21 CFR 312.35(b) ☐ TREATMENT PROTOCOL 21 CFR 312.35(a) ☐ CHARGE REQUEST/NOTIFICATION 21 CFR312.7(d)

FOR FDA USE ONLY

CDR/DBIND/DGD RECEIPT STAMP	DDR RECEIPT STAMP	DIVISION ASSIGNMENT:
		IND NUMBER ASSIGNED:

12. **CONTENTS OF APPLICATION**

This application contains the following items: *(Check all that apply)*

- ☐ 1. Form FDA 1571 *[21 CFR 312.23(a)(1)]*
- ☐ 2. Table of Contents *[21 CFR 312.23(a)(2)]*
- ☐ 3. Introductory statement *[21 CFR 312.23(a)(3)]*
- ☐ 4. General Investigational plan *[21 CFR 312.23(a)(3)]*
- ☐ 5. Investigator's brochure *[21 CFR 312.23(a)(5)]*
- ☐ 6. Protocol(s) *[21 CFR 312.23(a)(6)]*
  - ☐ a. Study protocol(s) *[21 CFR 312.23(a)(6)]*
  - ☐ b. Investigator data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - ☐ c. Facilities data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - ☐ d. Institutional Review Board data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
- ☐ 7. Chemistry, manufacturing, and control data *[21 CFR 312.23(a)(7)]*
  - ☐ Environmental assessment or claim for exclusion *[21 CFR 312.23(a)(7)(iv)(e)]*
- ☐ 8. Pharmacology and toxicology data *[21 CFR 312.23(a)(8)]*
- ☐ 9. Previous human experience *[21 CFR 312.23(a)(9)]*
- ☐ 10. Additional information *[21 CFR 312.23(a)(10)]*

13. IS ANY PART OF THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRACT RESEARCH ORGANIZATION? ☐ YES ☐ NO

IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CONTRACT RESEARCH ORGANIZATION? ☐ YES ☐ NO

IF YES, ATTACH A STATEMENT CONTAINING THE NAME AND ADDRESS OF THE CONTRACT RESEARCH ORGANIZATION, IDENTIFICATION OF THE CLINICAL STUDY, AND A LISTING OF THE OBLIGATIONS TRANSFERRED.

14. NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE CONDUCT AND PROGRESS OF THE CLINICAL INVESTIGATIONS

15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW AND EVALUATION OF INFORMATION RELEVANT TO THE SAFETY OF THE DRUG

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set fourth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

16. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE	17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE	
18. ADDRESS *(Number, Street, City, State and Zip Code)*	19. TELEPHONE NUMBER *(Include Area Code)*	20. DATE

(WARNING: A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Food and Drug Administration
CBER (HFM-99)
1401 Rockville Pike
Rockville, MD 20852-1448

Food and Drug Administration
CDER (HFD-94)
12229 Wilkins Avenue
Rockville, MD 20852

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please DO NOT RETURN this application to this address.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

STATEMENT OF INVESTIGATOR

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)

(See instructions on reverse side.)

Form Approved: OMB No. 0910-0014.
Expiration Date: January 31, 2006.
See OMB Statement on Reverse.

NOTE: No investigator may participate in an investigation until he/she provides the sponsor with a completed, signed Statement of Investigator, Form FDA 1572 (21 CFR 312.53(c)).

1. NAME AND ADDRESS OF INVESTIGATOR

2. EDUCATION, TRAINING, AND EXPERIENCE THAT QUALIFIES THE INVESTIGATOR AS AN EXPERT IN THE CLINICAL INVESTIGATION OF THE DRUG FOR THE USE UNDER INVESTIGATION. ONE OF THE FOLLOWING IS ATTACHED.

☐ CURRICULUM VITAE ☐ OTHER STATEMENT OF QUALIFICATIONS

3. NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED.

4. NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY.

5. NAME AND ADDRESS OF THE INSTITUTIONAL REVIEW BOARD (IRB) THAT IS RESPONSIBLE FOR REVIEW AND APPROVAL OF THE STUDY(IES).

6. NAMES OF THE SUBINVESTIGATORS *(e.g., research fellows, residents, associates)* WHO WILL BE ASSISTING THE INVESTIGATOR IN THE CONDUCT OF THE INVESTIGATION(S).

7. NAME AND CODE NUMBER, IF ANY, OF THE PROTOCOL(S) IN THE IND FOR THE STUDY(IES) TO BE CONDUCTED BY THE INVESTIGATOR.

PSC Media Arts (301) 443-1090 EF

8. ATTACH THE FOLLOWING CLINICAL PROTOCOL INFORMATION:

☐ FOR PHASE 1 INVESTIGATIONS, A GENERAL OUTLINE OF THE PLANNED INVESTIGATION INCLUDING THE ESTIMATED DURATION OF THE STUDY AND THE MAXIMUM NUMBER OF SUBJECTS THAT WILL BE INVOLVED.

☐ FOR PHASE 2 OR 3 INVESTIGATIONS, AN OUTLINE OF THE STUDY PROTOCOL INCLUDING AN APPROXIMATION OF THE NUMBER OF SUBJECTS TO BE TREATED WITH THE DRUG AND THE NUMBER TO BE EMPLOYED AS CONTROLS, IF ANY; THE CLINICAL USES TO BE INVESTIGATED; CHARACTERISTICS OF SUBJECTS BY AGE, SEX, AND CONDITION; THE KIND OF CLINICAL OBSERVATIONS AND LABORATORY TESTS TO BE CONDUCTED; THE ESTIMATED DURATION OF THE STUDY; AND COPIES OR A DESCRIPTION OF CASE REPORT FORMS TO BE USED.

9. COMMITMENTS:

I agree to conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

I agree to report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64.

I have read and understand the information in the investigator's brochure, including the potential risks and side effects of the drug.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

I agree to maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.

I will ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

INSTRUCTIONS FOR COMPLETING FORM FDA 1572
STATEMENT OF INVESTIGATOR:

1. Complete all sections. Attach a separate page if additional space is needed.
2. Attach curriculum vitae or other statement of qualifications as described in Section 2.
3. Attach protocol outline as described in Section 8.
4. Sign and date below.
5. FORWARD THE COMPLETED FORM AND ATTACHMENTS TO THE SPONSOR. The sponsor will incorporate this information along with other technical data into an Investigational New Drug Application (IND).

10. SIGNATURE OF INVESTIGATOR	11. DATE

(WARNING: A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

Please DO NOT RETURN this application to this address.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research

INFORMATION FOR SPONSOR-INVESTIGATORS SUBMITTING INVESTIGATIONAL NEW DRUG APPLICATIONS (INDs)

An Investigational New Drug Application (IND) is a request for Food and Drug Administration (FDA) authorization to administer an investigational drug to humans. Such authorization must be secured prior to interstate shipment and administration of any new drug that is not the subject of an approved new drug application.

IND regulations are contained in Title 21, Code of Federal Regulations, Part 312. Copies of the regulations, further guidance regarding IND procedures, and additional forms are available from the FDA Center for Drug Evaluation and Research, Drug Information Branch (HFD-210), 5600 Fishers Lane, Rockville, Maryland 20857, telephone (301) 827-4573 or toll free at 1-888-INFOFDA. In addition, forms, regulations, guidances, and a wide variety of additional information is available online at http://www.fda.gov/cder/. Forms may be accessed directly at either http://www.fda.gov/opacom/morechoices/fdaforms/cder.html or http://forms.psc.gov/forms/FDA/fda.html

The following instructions address only the administrative aspects of preparing and submitting an IND, and are intended primarily to provide assistance to individual Sponsor-Investigator applicants, not pharmaceutical companies.

WHERE TO SEND THE APPLICATION:

The initial IND submission and each subsequent submission to the IND should be accompanied by a Form FDA 1571 and must be submitted in triplicate (the original and two photocopies are acceptable). Mailing addresses for initial IND submissions are:

For a Drug:

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
12229 Wilkins Ave.
Rockville, MD 20852-1833

For a Biologic:

Food and Drug Administration
Center for Biologics Evaluation and Research, HFM-99, Rm 200N
1401 Rockville Pike
Rockville, MD 20852-1448

FILLING OUT THE FORM FDA 1571: (The numbers below correspond to the numbered boxes on the Form FDA 1571.)

1. The sponsor is the person who takes responsibility for and initiates a clinical investigation. The sponsor may be a pharmaceutical company, a private or academic organization, or an individual. **A Sponsor-Investigator is an individual who both initiates and conducts a clinical investigation and under whose immediate**

direction the investigational drug is being administered or dispensed. For administrative reasons, only one individual should be designated as sponsor.

If a pharmaceutical company will be supplying the drug, but will not itself be submitting the IND, the company is not the sponsor.

2. The date of submission is the date that the application is mailed to FDA.

3. The address is the address to which written correspondence from FDA should be directed. If this address is a post office box number, a street address must also be provided.

4. The telephone number is the number where the sponsor is usually available during normal working hours. A telephone number must be provided.

5. For name(s) of drug, list the generic name(s) and trade name, if available. Also, state the dosage form(s).

6. If an emergency IND number was previously assigned by FDA, or the Form FDA 1571 is being included with an amendment to the original IND, then that IND number should be entered here; otherwise, the space should be left blank.

7. Self-explanatory.

8. This section is to be completed by pharmaceutical firms that are conducting clinical studies in support of a marketing application. Sponsor-Investigators need not complete this section.

9. It is necessary for the sponsor to submit certain information with an IND (such as manufacturing and controls information, pharmacology and toxicology data, or data from prior human studies) unless that information has previously been submitted to FDA, AND the sponsor of the previously submitted information provides a letter authorizing FDA to refer to the information. In this case, the letter of authorization including the file identification (IND/DMF/NDA number) must be: 1) submitted to the authorizer's application and, 2) included in the initial submission of the new sponsor's IND. The sole exception to this requirement is when a marketed drug is used in the study, without modification to its approved packaging, in which case the marketed drug product must be identified by trade name, established name, dosage form, strength, and lot number.

10. Numbering of submissions is primarily intended for pharmaceutical firms. Sponsor-Investigators do not have to complete this section.

11. For an original IND submission, only the "Initial Investigational New Drug Application (IND)" box should be checked. For subsequent submissions, check ALL the boxes that apply since the submission may contain more than one type of information.

Requests to charge and Treatment Protocols must be submitted separately. Treatment INDs and Treatment Protocols are special cases and are not intended

for single patient use. Before checking either of these boxes, the sponsor should be thoroughly familiar with the cited regulations and contact the appropriate FDA reviewing division to discuss the proposed treatment use.

12. For a Sponsor-Investigator IND, items 2, 3, and 4 may be briefly addressed in the cover letter or in a summary.

Where the investigational drug is obtained from a supplier in a final dosage form, items 5, 7, 8, and 9 may be referenced if authorization is given by the supplier (see explanation in section 9 above). If the investigational drug is prepared or altered in any way after shipment by the supplier, complete manufacturing (or compounding) and controls information, including information on sterility and pyrogenicity testing for parenteral drugs, must be submitted for that process in Item 7.

Item 6 requires that the protocol be submitted, along with information on the investigators, facilities, and Institutional Review Board (copies of the completed Form FDA 1572 with attachments would suffice for 6 b-d).

Item 7 also requires submission of either a claim of categorical exclusion from the requirement to submit an environmental assessment or an environmental assessment (21 CFR 25.15[a]). When claiming a categorical exclusion, the sponsor should include the following statements: "I claim categorical exclusion (under 21 CFR 25.31[e]) for the study(ies) under this IND. To my knowledge, no extraordinary circumstances exist."

13. This section does not pertain to a Sponsor-Investigator.

14-15. For a pharmaceutical firm, the name of the person responsible for monitoring the conduct of the clinical investigation, and reviewing and evaluating safety information, should be entered. For Sponsor-Investigator INDs, the investigator has this responsibility.

N.B. Certain important commitments that the IND sponsor makes by signing the form FDA 1571 are listed below box 15.

16-17. For an IND sponsored by a pharmaceutical firm or research organization, the name of the sponsor's authorizing representative would be entered and that individual must sign the form For a Sponsor-Investigator IND, the Sponsor-Investigator should be named and must sign the form.

18-19. Box 18 and 19 need not be completed if they duplicate boxes 3 and 4.

20. The date here is the date the form is signed by the sponsor.

FORM FDA 1572:

Copies of Form FDA 1572 with its attachments may be sent by the Sponsor-Investigator to FDA to satisfy Form FDA 1571, box 12, item 6 b-d. Information can be supplied in the form of attachments (such as a curriculum vitae) rather than entering that information

directly onto the form, but this should be so noted under the relevant section numbers.

FDA RECEIPT OF THE IND:

Upon receipt of the IND by FDA, an IND number will be assigned, and the application will be forwarded to the appropriate reviewing division. The reviewing division will send a letter to the Sponsor-Investigator providing notification of the IND number assigned, date of receipt of the original application, address where future submissions to the IND should be sent, and the name and telephone number of the FDA person to whom questions about the application should be directed. Studies shall not be initiated until 30 days after the date of receipt of the IND by FDA unless you receive earlier notification by FDA that studies may begin.

[12/09/1998]

Center for Drug Evaluation and Research
Page Updated: March 08, 2001

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Exhibit I

Letter of August 12, 2009, submitting BLA 125363 to FDA

August 12, 2009

Norman Baylor, Ph.D., Director
Office of Vaccines Research and Review
Center for Biologics Evaluation and Research, Suite 200N
Food and Drug Administration
HFM-99
1401 Rockville Pike,
Rockville, MD 20852-1448

Re: STN: BL 125363; MENHIBRIX (Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine)
US License No. 1617
Original Submission
Sequence No: 0000

Attention: J. Temenak, PhD; HFM-481

Dear Dr. Baylor:

Provided herein is the Biologics License Application (BLA) in electronic Common Technical Document (eCTD) format for GlaxoSmithKline (GSK) Biologicals' Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine. Clinical development of this vaccine, which was originally designated Hib-MenCY-TT, was conducted under US IND 11706. The development program for Hib-MenCY-TT was granted Fast Track designation on January 24, 2005.

The proposed proprietary name for this vaccine is MenHibrix®. An initial request for review of this proprietary name was submitted to IND 11706 on April 7, 2008. Additional information in response to CBER request was submitted to the IND on July 08, 2009. The documents provided in these IND amendments are also included in the enclosed BLA.

MenHibrix is a non infectious vaccine that contains *Neisseria meningitidis* serogroup C capsular polysaccharide (PSC), *Neisseria meningitidis* serogroup Y capsular polysaccharide (PSY), and *Haemophilus influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate, PRP), each covalently bound to tetanus toxoid. The vaccine formulation is a lyophilized product supplied in a 3 mL monodose glass container (Type I), stoppered with rubber closures for lyophilization and closed with flip-off caps. The vaccine is to be reconstituted prior to intramuscular injection, with a liquid saline diluent supplied in pre-filled syringes containing 0.7 mL of diluent. The reconstituted product contains 2.5 μg of PRP-TT, 5 μg PSC-TT and 5 μg PSY-TT per 0.5 mL dose volume.

The proposed indication is for active immunization of infants and toddlers 6 weeks through 15 months of age for the prevention of invasive diseases caused by *Neisseria meningitidis* serogroups C and Y and *Haemophilus influenzae* type b.

The clinical package to support licensure of *MenHibrix* consists of 13 completed Phase II and III clinical studies. These studies were conducted in the United States (US), Australia, Belgium, Germany, and Mexico. The submission also includes safety data obtained in two ongoing studies, both conducted in the US.

User Fee Information
A fee in the amount of $1,247,200.00 has been paid in full for the review of this application. The User Fee Cover Sheet (Form FDA 3397) is provided in Module 1.1.3, along with proof of remittance (User Fee ID No. PD3009525 has been assigned to this application).

BLA Form
The completed Form FDA 356h Application to Market a New Drug, Biologic, or Antibiotic Drug for Human Use is provided in Module 1.1.2. CBER has assigned STN BL 125363 to this BLA.

Authorized Agent Information
The primary authorized representative for this application is Jody Ann Gould, PhD, Director, North American Regulatory Affairs.

Additional authorized representatives are as follows:
Elisa Harkins, Associate Director, North American Regulatory Affairs.
Linda Kramer, Associate Director, North American Regulatory Affairs, Establishments
Norris Pyle, Assistant Director, North American Regulatory Affairs, CMC
Teresa Ward, RAC, Senior Director, North American Regulatory Affairs, Establishments

All authorized representatives for this application are located at the following address:
GlaxoSmithKline
2301 Renaissance Boulevard, Building 510
P.O. Box 61540
King of Prussia, PA 19406-2772

Direct contact information for all authorized representatives is provided below:

Name	*Phone*	*Email*	*Fax*
Jody Ann Gould	610-787-3765	jody.a.gould@gsk.com	610-787-7063
Elisa Harkins	610-787-3601	elisa.b.harkins@gsk.com	610-787-7063
Linda Kramer	610-787-3151	linda.s.kramer@gsk.com	610-787-7063
Norris Pyle	610-787-3755	norris.h.pyle@gsk.com	610-787-7063
Teresa Ward	610-787-3769	teresa.2.ward@gsk.com	610-787-7063

Pediatric Use

The present application provides information to support the use of *MenHibrix* in infants and toddlers, from 6 weeks through 15 months of age. GSK is respectfully requesting a waiver of PREA requirements for children 0 to < 6 weeks of age and from 16 months to 17 years of age (prior to 18th birthday). GSK acknowledges CBER's comment (June 18, 2009) that clinical development of *MenHibrix* may be desirable for pediatric subjects 15 months to < 18 years old in the event that outbreak control is needed and for immunization of older children at high risk for meningococcal disease. Consideration of such development should also take into account the recommendations for Hib vaccination, as well as the feasibility of the studies. Based on these considerations, GSK requests a waiver from the PREA requirement for the 16 month to < 18 year old age range. The complete Request for Waiver of PREA Requirements is provided in Module 1.9.1.

Request for Priority Review

GSK is respectfully requesting that CBER grant priority status to the review of this BLA. The clinical development program for *MenHibrix* has had Fast Track designation since January 2005. To date, no vaccine has been approved for the prevention of meningococcal disease in persons less than 2 years of age. As outlined in the complete Request for Priority Review (Module 1.2), *MenHibrix* continues to meet the requirements for Fast Track designation, and, therefore, meets the requirements for Priority Review.

Labeling

Module 1.14 includes the proposed package insert (PI) and proposed packaging components for *MenHibrix*. Where applicable, we have incorporated CBER's feedback and agreements regarding the PI for *Hiberix* (STN BL 125347; currently under review) to the proposed PI for *MenHibrix*. This includes CBER's request to add information regarding the amount of residual formaldehyde to the PI. The calculation of the amount of residual formaldehyde for *MenHibrix* is provided in Module 1.11.1.

Please note that two sets of packaging component labeling (i.e. carton, vial, and syringe labels) are provided. One set applies to vaccine packaged at GSK's site in Wavre, Belgium, currently listed in the BLA. The additional set will apply to packaging performed at GSK's site in Marietta, Pennsylvania. These components are included in the BLA for CBER's review in anticipation of adding Marietta as an alternative packaging site for *MenHibrix* following approval of the Marietta site and of the *MenHibrix* BLA.

Included in this submission is our request of UNII (unique ingredient identifier) codes for *MenHibrix*. This request is provided in Module 1.2.

BLA Content and Format

This application is provided in electronic format only and in compliance with "*Guidance for Industry – Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications*" (June 2008). Please refer to the Guide to the FDA Reviewer provided in Module 1.2 for complete details on the electronic submission.

We have incorporated into the BLA analyses requested by CBER and agreed by GSK and CBER; in particular, we have included information and analyses as discussed or described in following communications: March 12, 2009 CBER fax; March 13, 2009 teleconference; March 23, 2009 teleconference; May 19, 2009 CBER email; June 18, 2009 CBER fax; June 19, 2009 Type B Pre-BLA teleconference; June 19, 2009 CBER faxes; July 10, 2009 CBER fax.

GSK would like to note the following regarding the contents of this application:

- The polyribosyl-ribitol-phosphate tetanus toxoid conjugate is referred to as PRP-TT and Hib-TT interchangeably throughout the BLA.
- The proposed tradename for the vaccine is *MenHibrix* and the proposed generic name is 'Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine'. Within the BLA, the vaccine may be referred to as *Haemophilus influenzae* type b and *Neisseria meningitidis* Serogroups C and Y – Tetanus Toxoid Conjugate Vaccine or as Hib-MenCY-TT, for consistency with the IND, previously submitted study reports and other documents, and previous communications with CBER.
- For some studies, the Case Report Forms (CRFs) provided in the BLA may be different than those submitted to the IND. This difference is due to an update of CRFs to include information across all 4 doses for the BLA.
- This BLA submission includes an integrated safety analysis dataset ('ISS dataset') with safety information pooled from studies Hib-MenCY-TT-001, 003/004, 005/006, 007/008, 009/010 and 011/012, and a separate dataset for the ongoing study MenACWY-TT-057. The Summary of Clinical Safety (m.2.7.4) describes the results of safety analyses performed on the ISS dataset. The meta-analysis requested by CBER to justify the pooling of safety data in the ISS was performed and has been included in m2.7.4.
- Per CBER's request, GSK has conducted analyses of safety data across all 4 doses of the vaccine. The BLA includes an annex to the reports for studies Hib-MenCY-TT-009/010 and 011/012 that summarizes pooled safety data for these studies across the 4 dose series. As discussed and agreed at the June 19, 2009 pre-BLA teleconference, annex reports that include analyses of safety data across all 4 doses for individual studies (Hib-MenCY-TT-005/006, 007/008, 009/010 and 011/012) will be submitted within 1 month of the initial BLA.

- Per CBER's request, sensitivity analyses to evaluate the potential impact of excluding data from a single US study site (identified as site number 24660 in study Hib-MenCY-TT-009, site number 34932 in study Hib-MenCY-TT-010, site number 35785 in study Hib-MenCY-TT-011, and site number 39451 in study Hib-MenCY-TT-012) have been performed. The results of the sensitivity analyses for both immunogenicity and safety data are provided in the reports for studies Hib-MenCY-TT-009 and 010 as per CBER request.
- Datasets for individual studies Hib-MenCY-TT-005, 006, 007/008, 009/010 and 011/012 are also provided. For study Hib-MenCY-TT-009/010, GSK has incorporated requested information related to serology tests performed into the database.
- Per CBER's request, an analysis of the responses to the antigens in *Prevnar* antigens using a threshold of 0.35 μg/mL has been included for reference purposes. The results of this analysis are provided in the annex to the report for study Hib-MenCY-TT-005.
- Per CBER's request, an analysis of the response to the 4th dose of *MenHibrix* in study Hib-MenCY-TT-008 is provided in an annex to the report for that study.
- Per CBER's request, standard operating procedures, validation protocols and validation reports for drug substance release and drug product release are being provided (Module 3.2.R).

If you have any questions, or require any additional information, please do not hesitate to contact me by phone at 610-787-3765, by fax at 610-787-7063 or by email at jody.a.gould@gsk.com.

Sincerely,

Jody Ann Gould, Ph.D.
Director
Regulatory Affairs

Trade secret and/or confidential commercial information contained in this submission is exempt from public disclosure to the full extent provided under law.

Exhibit J

Chronology of Regulatory Review of MENHIBRIX™

Chronology of Regulatory Review for MENHIBRIX™	
Date	**Description**
April 9, 2003	pre-IND meeting with FDA
May 12, 2004	**GSK submits initial IND (BB-IND No. 11,706)**
May 14, 2004	FDA receipt of IND submission
June 12, 2004	**IND in effect**
July 12, 2004	FDA letter requesting clinical and CMC information
August 13, 2004	First U.S. phase II clinical study initiated
November 22, 2004	IND amendment submitted to FDA (Draft Protocol)
January 24, 2005	MENHIBRIX™ designated as a Fast Track Development program
February 4, 2005	Teleconference with FDA regarding clinical design
February 8, 2005	GSK letter to FDA responding to FDA request for comments on clinical design
April 12, 2005	FDA letter providing comments on immunogenicity study protocol
May 10, 2005	IND amendments submitted to FDA (pre-phase III briefing document)
June 8, 2005	Pre-Phase 3 teleconference with FDA
July 1, 2005	Additional U.S. phase II clinical study at additional study centers initiated
September 1, 2005	IND amendment submitted to FDA
September 26, 2005	Teleconference with FDA (FDA feedback on GSK's responses to FDA's questions regarding study design)
October 27, 2005	Type A meeting with FDA to discuss expanded primary endpoints and secondary endpoints
December 20, 2005	IND amendments submitted to FDA (endpoint criteria for co-administration study)
February 22, 2006	Primary vaccination study (phase III) initiated
February 27, 2006	Teleconference with FDA (clinical study modifications)
March 20, 2006	Teleconference with FDA (clarification of planned clinical study modifications)
March 29, 2006	First phase II study completed
May 8, 2006	Additional phase II study completed
May 30, 2006	FDA notifies GSK that immunogenicity endpoints based on hSBA assay are acceptable for licensure
June 2, 2006	Teleconference with FDA (regarding US immunogenicity study)
July 27, 2006	IND amendments submitted to FDA
August 4, 2006	Teleconference with FDA (criteria for assessing vaccine lot consistency and immunogenicity analyses)
August 30, 2006	Teleconference with FDA (discuss booster phase of study)
September 11, 2006	First persistence study (phase II) initiated
September 15, 2006	Phase III comparative safety study (vs. monovalent PRP) initiated
October 18, 2006	FDA letter providing comments on immunogenicity and safety studies
December 19, 2006	IND amendment (clinical), SOPs, and serology assay validation reports submitted to FDA
December 29, 2006	Fourth dose vaccination study (phase III) initiated
February 14, 2007	Teleconference with FDA to discuss clinical and safety data
February 27, 2007	IND amendment submitted to FDA (immunogenicity study protocol changes)
May 10, 2007	IND amendments submitted to FDA (clinical and statistical information)
June 6, 2007	IND amendments submitted to FDA (clinical and statistical information)
July 6, 2007	FDA fax requesting comments and information regarding microbiology
September 20, 2007	IND amendments submitted to FDA (protocol amendment: revised investigators)
October 4, 2007	IND amendments submitted to FDA (clinical and ctatistical information)

November 28, 2007	First persistence study completed
December 18, 2007	Teleconference with FDA (clinical information)
January 25, 2008	IND amendments submitted to FDA (reporting and analysis plans)
February 26, 2008	Primary vaccination study completed
March 7, 2008	IND amendment (response to FDA comments regarding hSBA assay and assay validation)
April 3, 2008	Additional persistence study (phase II) initiated
May 13, 2008	Teleconference with FDA (clinical, protocol, statistical, and phase 3 immunogenicity endpoints)
June 30, 2008	FDA fax to GSK with questions regarding hSBA assay and assay validation
September 4, 2008	GSK requests Type A meeting with FDA
October 8, 2008	Teleconference with FDA
October 20, 2008	IND amendment submitted to FDA (additional information submitted in response to teleconference of October 8, 2008)
November 12, 2008	Phase III comparative safety study (vs. monovalent PRP) completed
December 18, 2008	IND amendment submitted to FDA (final serology SOPs incorporating changes described in prior IND amendments and responsive to prior discussions with FDA)
January 13, 2009	Additional IND amendments submitted
March 13, 2009	Teleconference with FDA (final agreement regarding revised immunogenicity endpoints)
March 20, 2009	Amendments to the Report and Analysis Plans provided to FDA by email
March 23, 2009	Agreement reached with FDA on primary statistical analyses
April 7, 2009	Amendments to the Report and Analysis Plans formally submitted to FDA
May 22, 2009	IND amendment submitted to FDA (Briefing Document for Type B Pre-BLA Meeting)
May 29, 2009	Additional persistence study completed
June 19, 2009	Pre-BLA meeting with FDA
August 12, 2009	**GSK submits BLA for MENHIBRIX™**
June 10, 2010	Supplemental persistence study (phase II) initiated
May 12, 2011	Supplemental persistence study completed
September 23, 2011	GSK receives Complete Response letter from FDA
June 14, 2012	**MENHIBRIX™ BLA approved**